Bacterial growth and grazing loss in contrasting areas of North and South Atlantic

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Abstract. Samples were collected from the top 200 m of the water column at 50 stations during two cruises in different, near equinoctial seasons on an Atlantic transect near the 20°W meridian between 50°N and 50°S. These samples were analysed to determine characteristics of the heterotrophic bacterial populations. Flow cytometry was used to enumerate these bacteria and determine their average size so as to calculate their biomass. Heterotrophic bacterial production, and the rate of grazing of these bacteria by heterotrophic nanoplankton in the main depth layers, were determined using 3H thymidine and 14C leucine techniques. The biomass of heterotrophic nanoplankton in these layers was determined using a glucosaminidase assay. Five provinces were distinguished along the transect and characterized by average values of all measured parameters. The relative composition and activity of the microbial community in the water columns within each province changed little between the two cruises. Lowest heterotrophic bacterial biomass of 1–2 mg C m–3 and production of 0.1–0.2 mg C m–3 day–1 were found in the northern and southern Atlantic gyres, and were relatively similar in both seasons. Biomass and production were 2–4 times higher in the northern and southern temperate waters, and in equatorial waters, than in the gyres and tended to show more seasonal variation. Production and biomass in the layer below the pycnocline were lower by 10–30% and about 50%, respectively, than values determined in the surface mixed layer, and varied less with latitude. Depth-integrated values of these two parameters were generally of similar size in the mixed water layer and the layer of the chlorophyll maximum and pycnocline, and tended to vary with season. The specific growth rate of heterotrophic bacteria was in the range 0.05 to 0.12 day–1 in the top mixed layer at all latitudes. In spite of the elevated temperatures, bacterial growth appears to be restricted by a shortage of nutrients so that the microbial community cycles very slowly, with a turnover time of the order of 1 week or more. The depth-integrated biomass of heterotrophic nanoplankton was generally about 100% of the heterotrophic bacterial biomass in the same water. Grazing by these nanoplankton at the rate measured could consume all of the new production of heterotrophic bacteria in all waters, and they probably control the populations of both heterotrophic and phototrophic bacteria.

Introduction

Ubiquitous heterotrophic bacteria dominate the mineralization of dissolved organic matter (DOM) in the sea (Azam and Hodson, 1977). In the open ocean, at a macroscale, they are largely reliant on phytoplankton for their principal energy supply. It is generally assumed that in the stratified pelagial, primary production is sustained more by the recycling of the growth-limiting nutrients than by the diffusion of nutrients through the pycnocline. Heterotrophic bacteria have been considered major sources of nutrients released through nanoplankton grazing in the microbial food web [e.g. (Pomeroy, 1974; Azam et al., 1983;
Bacterivorous heterotrophic nanoplankton channels the energy derived from the digestion of bacterial biomass up the food chain (Stoecker and Capuzzo, 1990) and releases metabolic by-products, partly as inorganic nutrients [e.g. (Andersson et al., 1985; Caron and Goldman, 1990; Ferrier-Pagès and Rassoulzadegan, 1994)], which can be directly utilized by algae in surface nutrient-depleted waters. The development of techniques which allow quantification of bacterial abundance (Hobbie et al., 1977; Porter and Feig, 1980) and production (Fuhrman and Azam, 1982) has provided tools and therefore stimulated a large number of studies on the carbon flux through the microbial loop.

In general, there is much better understanding of the functioning of planktonic communities associated with eutrophic and mesotrophic waters than with oligotrophic waters, usually because of a lack of information about the latter. It was believed that heterotrophic bacteria are the dominant component of oligotrophic ocean ecosystems [e.g. (Fuhrman et al., 1989; Cho and Azam, 1990)]. The recent discovery of Prochlorococcus cyanobacteria (Chisholm et al., 1988) completely changed the prevailing views on the composition of phytoplankton in oligotrophic waters. Comprehensive studies have been made in recent years on the picophytoplankton of some oligotrophic regions of both the Pacific Ocean [e.g. (Campbell and Vaulot, 1993; Campbell et al., 1994, 1997; Vaulot et al., 1995)] and the Atlantic Ocean [e.g. (Li, 1995; Buck et al., 1996; Partensky et al., 1996)]. The prokaryotic fraction of the planktonic community is composed of taxonomically diverse heterotrophic bacteria (Giovannoni et al., 1990) and the closely related phototrophic cyanobacteria (Palenik, 1994), Prochlorococcus spp. (Chisholm et al., 1988) and Synechococcus spp. (Waterbury et al., 1979). These groups of cyanobacteria are easily discriminated by flow cytometry due to their different pigment composition (Olson et al., 1993). Heterotrophic bacteria are generally enumerated by epifluorescence microscopy (Hobbie et al., 1977; Porter and Feig, 1980). However, Prochlorococcus cells, whose autofluorescence fades rapidly, cannot be distinguished from heterotrophic bacteria on slides (Sieracki et al., 1995) and therefore, the abundance of heterotrophic bacteria would be overestimated by inclusion of faded Prochlorococcus. This overestimation is especially significant in oligotrophic waters which have a high abundance of Prochlorococcus. Therefore, heterotrophic bacterial abundance and growth rates in oligotrophic regions of the oceans need to be re-evaluated (Campbell et al., 1994). Recently, several dyes were introduced for flow cytometric enumeration and characterization of prokaryotic cells (Monger and Landry, 1993; Li et al., 1995; Marie et al., 1997) and we combined flow cytometric analysis of unstained and stained samples to estimate abundance of heterotrophic bacteria and Prochlorococcus (Zubkov et al., 1998a, 1999a).

There is still little quantitative information about the trophic link between bacteria and protists in oligotrophic oceanic waters (Hagström et al., 1988; Caron et al., 1999). Meanwhile, there are numerous reports that bacterivorous protozoa can control bacterial concentrations in more productive waters, both marine (Wikner et al., 1986; MacManus and Fuhrman, 1988; Sherr et al., 1989;
Kuuppo-Leinikki, 1990; Weisse and Scheffel-Möser, 1991; Vaqué et al., 1994; Leakey et al., 1996) and limnic [e.g. (Pace, 1988; Bloem et al., 1989; Sanders et al., 1992)], consuming in the order of 25 to >100% of bacterial production per day. Although a wide range of methodological approaches has been employed to measure bacterivory [(Caron et al., 1993; Landry, 1994; Sherr and Sherr, 1994) and references therein], there are still great difficulties in obtaining sufficient sensitivity to apply them to oligotrophic waters. Recently, we developed a method to estimate directly and simultaneously the rates of grazing and assimilation of bacterial biomass into protists using a dual, radioactive-labelled bacterium, *Vibrio natriegens*, and subsequently, dual, radiolabelled, natural bacterioplankton, as food organisms (Zubkov and Sleigh, 1995, 1997; Zubkov et al., 1998b). There is a limited number of methods for estimating the biomass of heterotrophic nanoplanckton, the major bacterivores, and most of them require extensive microscopical study (Sherr et al., 1993). An alternative method based on the measurement of specific glucosaminidase activity was developed and recommended for use in the routine estimation of the biomass of heterotrophic nanoplanckton (Zubkov and Sleigh, 1998).

The aim of this study was to compare the standing stock and turnover of heterotrophic bacterioplankton in a wide range of stratified tropical and temperate oceanic waters of the Atlantic Ocean using some improved techniques: flow cytometry for counting picoplankton, pulse-chase dual radioactive labelling of natural heterotrophic bacteria for estimation of bacterivory, and glucosaminidase assay for estimation of the biomass of heterotrophic nanoplanckton. In particular, the refined techniques permit a reconsideration of the balance between bacterial production and grazing.

**Method**

**Sampling**

The work was conducted on cruises JCR15 and JCR21 of the R.R.S. James Clark Ross along the Atlantic Meridional Transect, from the British Isles to the Falkland Islands from 22 September to 25 October, 1996 (AMT-3), and in the opposite direction from 22 April to 26 May, 1997 (AMT-4). At intervals of about 4° latitude, sea water was collected with a cassette of Niskin bottles by CTD casts through the upper 200 m. Ten depths were usually sampled at the 23 stations on AMT-3, and usually 12 depths at the 27 stations on AMT-4 (Figure 1). Samples of equal volumes from 2–5 depths were pooled to model three main layers of the surface waters: first, the top mixed layer (L.1); second, the layer of the pycnocline and, where present, the deep chlorophyll maximum (L.2); third, the underlying top aphotic layer below the pycnocline (L.3) (Figure 2).

**Biomass measurements**

The concentrations and size of heterotrophic bacteria were measured by flow cytometry as described in more detail elsewhere (Zubkov et al., 1998a, 1999a). Briefly, 1.8 ml samples were fixed with 0.1–0.3% glutaraldehyde and kept below...
–30°C before being analysed. The total concentration of bacteria was measured in samples stained with a fluorochrome which has strong binding affinities to double-stranded DNA; TOTO-1 iodide (Li et al., 1995) was used on AMT-3 and SYBR Green 1 (Marie et al., 1997) on AMT-4. In general, the concentration of Prochlorococcus and Synechococcus was measured in unstained samples using group-specific side scatter, and orange and red autofluorescence properties, respectively (Olson et al., 1993). In order to estimate the numbers of heterotrophic bacteria, the numbers of Prochlorococcus and Synechococcus cyanobacteria measured in the same but unstained samples were subtracted from the total number of bacteria counted in stained samples. Fractionation through Nuclepore filters of different pore size, 0.4, 0.6, 0.8, 1.0 and 1.2 µm, was used to estimate an average size of heterotrophic bacteria, 0.46 ± 0.14 µm (n = 60). Using a cell density of 0.12 pg C µm⁻³ [mean of values given by (Christian and Karl, 1992)].

**Fig. 1.** The transect track showing the locations of sampling sites on AMT-3 (open triangles) and AMT-4 (filled triangles) cruises and five recognized provinces: northern temperate waters (NT), northern gyre (NG), equatorial region (EQ), southern gyre (SG), southern temperate waters (ST).
A value of 7 fg C per heterotrophic bacterium is obtained which is used throughout this paper.

The biomass of heterotrophic nanoplankton (HNP) was estimated by a glucosaminidase assay (Zubkov and Sleigh, 1998). Briefly, the fluorogenic substrate 4-methylumbelliferyl-n-acetyl-β-D-glucosaminide was used to assay the bacterial dynamics in Atlantic waters.
activity of lysosomal β-D-glucosaminidase (β-GAM) at acidic pH as an increase of the concentration of the released fluorochrome, 4-methyl-umbelliferone (MUF) with time. The total and particulate enzyme activity was measured. The samples were first screened through 60 µm mesh to remove metazoa and then 50 or 100 ml volumes were gravity-filtered through either GF/A or GF/C filters. The filter was immersed in a tube containing equal volumes (5 ml) of the original sample and 0.1 M acetic buffer, pH 4.6, with 0.2% 23 Lauryl ether (Brij 35). The substrate was added to reach a final concentration of 40 µM. The incubations were done in the dark at the in situ temperature of the mixed layer at stations where samples were collected, and sub-samples were fixed by 2:1 mixing with 0.4 M glycine-NaOH buffer, pH 11.2, after incubation for 0, 12 and 24 h. Fluorescence intensity was measured with an LS-3B fluorescence spectrometer (Perkin-Elmer Ltd, UK) at 370 nm excitation and 460 nm emission wavelengths. Fluorescence was calibrated initially against the concentration of MUF and subsequently, against the biomass of heterotrophic nanoplankton. To do this, the concentration and size of individual cells of heterotrophic nanoplankton in 21 samples of widely different trophic status along the AMT-3 transect were determined by DAPI direct counts (Sherr et al., 1993) under an epifluorescence microscope. Five sets of supplementary experiments were conducted in which the same samples were incubated in parallel at two temperatures, differing by 3–8.5°C, to derive a factor to correct rates of enzyme activity. In these experiments, \( R_t = R_i \times (1 + \Delta T \times 0.06) \), \( (n = 5, r^2 = 0.88) \), where \( R_t \) is the rate at ambient temperature, \( R_i \) is the rate at the temperature of incubation and \( \Delta T \) is the temperature difference (°C).

**Estimation of production of heterotrophic bacteria**

Production of bacterial cells and bacterial biomass was estimated from the simultaneous incorporation of \([3H]\)thymidine and \([14C]\)leucine (Chin-Leo and Kirchman, 1988). Samples (9 ml) from each layer studied were poured into each of five tubes, inoculated with [methyl-\( ^3 \)H]thymidine (50.0 Ci mmol\(^{-1}\), Amersham International plc., UK), 2.5 nM final concentration, and leucine L-[\( ^{14} \)C(U)] (350.2 Ci mol\(^{-1}\), DuPont NEN, Stevenage, UK), 25 nM final concentration, and incubated in the dark at the in situ temperature of the mixed layer at stations where samples were collected. One tube was used for each sample. A sample was fixed at 0 h and duplicated samples were fixed at 2 h and 4 h by mixing with 9 ml of 10% w/v trichloroacetic acid (TCA). Afterwards, the samples were filtered onto 0.2 µm polycarbonate filters and washed with 50 ml Milli-Q water. Any remaining traces of TCA were driven off by exposing filters at 60°C overnight. Radioactivity incorporated into TCA-insoluble material was counted with a Pharmacia (Turku, Finland) RackBeta 12809 liquid scintillation counter after several days of solubilization in Wallac (UK) Optiphase scintillation cocktail, using the installed three-over-two counting method for effective dual-label counting and the external standards ratio method. The rate of precursor incorporation was calculated as the slope of the linear regression of radioactivity against incubation time. As all incubations were made at the temperature of L.1, additional experiments were done to correct for temperature shift. Since there was no difference in temperature...
dependence between thymidine and leucine incorporation rates, an identical correction factor was used: \( R_t = R_i \times (1 + \Delta T \times 0.078) \), \( n = 10, r^2 = 0.94 \), where \( R_t \) is the rate at ambient temperature, \( R_i \) is the rate at the temperature of incubation and \( \Delta T \) is a temperature difference of more than 1°C. The relative concentrations of the two labelled precursors were chosen to fit the dual-label counting method.

Dilution culture experiments were conducted with surface mixed layer samples at five stations to estimate factors for converting the rates of precursor incorporation into bacterial production (Kirchman et al., 1982; Bjørnsen and Kuparinen, 1991). Replicated original samples (100 ml) were diluted 1:9 with 0.2 µm filtered sea water from the same site. Samples were kept in 1 l glass flasks in the dark at the in situ temperature of the surface mixed layer. The bottles were sub-sampled every 6 or 8 h for a total of 48 h to measure changes in the incorporation rates and bacterial abundance and size (biomass). The latter was estimated by size fractionation through 0.4, 0.6, 0.8 and 1 µm polycarbonate filters. Bacteria in the original sample and filtrates were enumerated by flow cytometry. The incorporation of precursors into bacteria in the sub-samples was performed according to the protocol described above, except that uptake was terminated at 0, 1 and 2 h. Conversion factors were calculated from a pooled dataset by the cumulative method (Bjørnsen and Kuparinen, 1991) as the slopes of linear regression of integrated substrate incorporation against bacterial yield during exponential growth: 0.8 ± 0.022 \( \times 10^{18} \) cells mol\(^{-1}\) thymidine \( (r^2 = 0.98, n = 13) \) and 235 ± 15 g C mol\(^{-1}\) leucine \( (r^2 = 0.92, n = 13) \). In order to compare estimates of production made by radioactive leucine and thymidine incorporation, the latter was multiplied by the estimated biomass of natural heterotrophic bacteria, 7 fg C cell\(^{-1}\).

Estimation of protozoan grazing on heterotrophic bacteria

Bacterivory was estimated using dual radioactive-labelled natural bacteria (Zubkov et al., 1998b). Briefly, experiments were based on pulse-chase labelling of natural bacteria and comparing their fate in bottles containing 100% of the normal grazer population with that in bottles containing 20% of these grazers.

Pulse-labelled heterotrophic bacteria were prepared by incubation with radiolabelled thymidine and leucine. After a standard incubation period, further uptake of isotopically-labelled compounds was reduced by adding a substantial excess of non-labelled thymidine and leucine before further incubation to allow the bacteria to reach a plateau in label uptake (Zubkov and Sleigh, 1995). These labelled bacteria in a medium containing low concentrations of labelled thymidine and leucine, and much higher concentrations of unlabelled thymidine and leucine, were then mixed with experimental sea-water samples containing unlabelled bacteria and protozoan predators. The unlabelled bacteria in these samples will show a small uptake of radiolabelled compounds, which will appear as a low background on filters. In order to estimate and subtract this low uptake, bacteria were labelled only at background level in parallel experiments by incubating them with labelled compounds in the presence of an excess level of non-labelled compounds.
For pulse-chase labelling of natural heterotrophic bacteria, 200 ml of the whole original sample (containing bacteria plus grazers) were inoculated with 2 nM of \(^3\)H-thymidine and 50 nM \(^{14}\)C-leucine (final concentrations) and incubated for 4 h. The sample was then inoculated with 2 \(\mu\)M and 30 \(\mu\)M of non-labelled thymidine and leucine, respectively, and subsequently incubated for 1 h before one volume of labelled sample was mixed either with 4 volumes of unlabelled original sample (100% grazers) or with 4 volumes of the same sample which had been passed through a 0.8 \(\mu\)m filter to remove grazers (20% grazers). The bottles were incubated in the dark and sub-sampled after 13 h as described below.

For background labelling of heterotrophic bacteria, 40 ml of original sample were inoculated with 2 nM of ‘cold’ thymidine and 50 nM of ‘cold’ leucine, incubated for 4 h, inoculated with 2 \(\mu\)M of thymidine mixture (2 nM \(^3\)H-thymidine and 2 \(\mu\)M cold thymidine) and 30 \(\mu\)M of leucine mixture (50 nM \(^{14}\)C-leucine and 30 \(\mu\)M of cold leucine), incubated for 1 h longer and dispensed similarly to pulse-labelled bacteria.

Unfixed samples from each type of bottle were filtered onto a (coarse) 0.8 or 1.2 \(\mu\)m polycarbonate filter and in parallel on a GF/A filter (10 \(\times\) 5 ml sub-samples were pooled and filtered at \(-0.02\) bar on a filter area of approximately 10 \(\text{cm}^2\)), and also onto a (fine) 0.2 \(\mu\)m polycarbonate filter (5 \(\times\) 5 ml sub-samples pooled and filtered at \(-0.2\) bar on a filter area of 2.5 \(\text{cm}^2\)). The filters were washed with equal volumes of 0.2 \(\mu\)m-filtered sea water. Differences between the ‘experimental’ (100% grazers) and ‘control’ (20% grazers) bottles in the retention of each of the labels on coarse filters, corrected for background labelling, were interpreted to indicate the extent of assimilation of radioactive-labelled compounds from bacteria by protozoa, and were related to the total amount of particulate radioactivity (that collected on fine filters).

**General methods**

All plastic- and glassware used for handling and incubation of sea-water samples was given a thorough preliminary wash with 1 N HCl and rinsed with 0.2 \(\mu\)m-filtered sea water. The grazing experiments were conducted in 250 ml polycarbonate bottles (Nalgene, Merck, UK). Experiments on incorporation of radioactive substrates by heterotrophic bacteria were performed in 50 ml polypropylene centrifuge tubes (Falcon, Becton-Dickinson, UK). All non-radioactive chemicals were purchased from Sigma.

**Statistical analysis**

Samples analysed by flow cytometry were always obtained by pooling several sub-samples. The accuracy of flow cytometric counting is well below 5%, and errors are therefore smaller than the size of symbols used on the graphs. The glucosaminidase measurements have an accuracy of about 5%. All grazing experiments were replicated. A \(t\)-test was used to compare the means, and linear regression was employed to compare datasets and to calculate uptake rates, supplemented with correlation analysis. All statistical tests were significant at
least at a 95% confidence limit. The numbers are presented as means ± SD, or as regression slopes ± SE.

Results

The design of AMT cruises gave an excellent opportunity for basin-scale comparative study of the microbial community of the Atlantic Ocean. However, it imposed some inevitable constraints. Thus, although 10 depths were sampled at most stations on AMT-3 and usually 12 depths on AMT-4, so that the spatial distribution of picoplankton was studied with adequate resolution by measurements made on every sample (Zubkov et al., 1998a, 1999a), the tight daily sampling routine restricted the number of measurements which could be made of those parameters which required incubations. A compromise was reached by adopting a layer approach for these measurements. Three layers were identified: the surface mixed water layer (L.1), the layer of seasonal pycnocline (which is also the nutricline in oligotrophic regions) including the deep chlorophyll maximum where present (L.2), and the top layer of the aphotic zone (L.3). The layer boundaries were chosen on the basis of the vertical distribution of temperature, water density and in situ fluorescence revealed by direct probing before discrete depths were selected and sampled (e.g. Figure 2). Samples which were collected from different depths but from the same water layer were pooled for measurements requiring incubations.

Comparison of the three defined layers

Contour plots of water density and temperature distribution along the transect showed similar general patterns on the two cruises (Figure 2). Deep surface mixed layers were characteristic for waters of oligotrophic gyres. The mixed layer was deeper in the southern gyre than the northern gyre. This layer was underlain by an extensive pycnocline (nutricline) layer, which in oligotrophic waters harboured concentrated phytoplankton that formed the deep chlorophyll maximum. Below these layers was a vast body of deep oceanic waters that were sampled on our cruises generally only down to 200 m. This deepest layer was the most homogeneous among the three layers. A stable frontal system seen on contour plots of temperature distribution formed a clear boundary between the southern temperate waters and waters of the southern gyre. Although there were obvious similarities of temperature and density profiles on the two cruises, there was a southwards progression of shallow stratified surface waters in the region near the equator on the AMT-4 (Figure 2b). This shift was a spectacular indicator of seasonal temperature changes in the Atlantic Ocean; cooler, less stratified waters in the North Atlantic which extend down towards the equator in the boreal spring should be expected to affect biological processes, including the dynamics of the microbial loop.

The concentration of heterotrophic bacteria in the upper layers, L.1 and L.2, was higher on AMT-4 than on AMT-3 and more similar and uniform during the two cruises in the deeper layer L.3 (Figure 3a). The biomass of heterotrophic
nanoplankton estimated using the enzymatic assay had similar distributions on the two cruises, except for a sharp increase in the mixed layer L.1 on AMT-3 in southern temperate waters in spring (Figure 3b). Also, higher concentrations were observed in the equatorial region on AMT-4, which were more extensive in the pycnocline layer, L.2, than the surface layer, L.1. Generally, the values of biomass on the two cruises were comparable in L.1 and L.2 at most of the stations, and for both classes of organism; they were two times higher in these upper layers than in the deeper layer, L.3.

The distribution of bacterial production estimated by uptake rates of $^{14}$C-leucine and $^3$H-thymidine along the transect also showed some differences between the two cruises (Figure 4). The major dissimilarities were observed in

![Figure 3](https://academic.oup.com/plankt/article-abstract/22/4/685/1468442/Bacterial-growth-and-grazing-loss-in-contrasting?intersiteToken=22d9d5c3-0954-4e7d-a65d-c20e5bb793e7)

**Fig. 3.** The distribution of biomasses of heterotrophic bacteria (a) and heterotrophic nanoplankton (b) in three layers (L.1, L.2, L.3) along the transect on AMT-3 and AMT-4 cruises.
the equatorial region. Although a spiky increase of production was measured on AMT-3 at about 20°N, in the vicinity of the Mauritanian upwelling, the rates on AMT-4 were higher in the whole area around the equator. The distribution of production was more temporally and spatially uniform in the deepest layer, L.3. In this layer, the rates were low at no more than one third of the rates in the layers above, although relatively high values were measured in L.3 at the two ends of the transect.

The values of bacterial production estimated using the two different precursors were closely similar in the two top layers on both cruises; the linear regression slopes were 0.76 and 0.77, respectively (Figure 5a). Low rates, well below 0.08 mg C m⁻³ day⁻¹, determined in the deepest layer, L.3, correlated poorly. A moderate

Fig. 4. The distribution of heterotrophic bacterial (HB) production estimated by simultaneous uptake of two precursors, ¹⁴C-leucine (a) and ³H-thymidine (b), in three layers (L.1, L.2, L.3) along the transect on AMT-3 and AMT-4 cruises.
Fig. 5. Inter-comparison in three layers (L.1, L.2, L.3) of (a) heterotrophic bacteria (HB) production estimated by uptake of two precursors, $^{14}$C-leucine and $^3$H-thymidine; (b) production and biomass of HB; (c) HB production and heterotrophic nanoplankton (HNP) biomass; and (d) HB and HNP biomasses. The open circles show results on AMT-3 and closed circles show results on AMT-4. Lines indicate linear regressions (except in the panel for L.3 in row d, where it is a unity line); heavy continuous lines show good correlation, thinner continuous lines moderate correlation, and dashed lines weak correlation; $r^2$ values are corresponding regression coefficients (see details in the text).
correlation between bacterial production and biomass \( (r^2 = 0.74) \) could be determined in the mixed layer, L.1 (Figure 5b), after the maximum value of bacterial biomass measured during the spring bloom in the northern temperate waters had been excluded from the dataset. Addition of this single value reduced the regression coefficient to 0.56. A weaker correlation with a similar slope of linear regression was found in the pycnocline layer, L.2. In the deepest layer, L.3, bacterial production decreased sharply and no significant correlation could be determined. The relation between bacterial production and the biomass of heterotrophic nanoplankton that fed on them was similar in the top two layers; it was more pronounced in the second layer, L.2, than in the mixed layer, L.1 (Figure 5c), and no correlation was found in the deep layer, L.3. The biomasses of potential bacterial prey and bacterivorous predators correlated weakly in the top two layers, and, as in the previous set of comparisons, no correlation was observed in the deep layer, L.3 (Figure 5d), although in this deep layer the points were more or less equally distributed above and below the line of unity.

Depth-integrated parameters clearly showed the trophic status of the transected oceanic waters and revealed the real contribution of each layer (Figure 6). The stocks of both bacteria and heterotrophic nanoplankton were lower in the southern and northern gyres and higher in areas close to the equator and the ends of the transect. The distinction between these regions was even more pronounced in distributions of bacterial production (Figure 6c, d). Therefore, we decided to proceed with analysis of our data using a province approach, considering that in a certain oceanic area, an average estimate of measured parameters can be obtained by calculating mean values from grouped stations. The boundaries separating the areas were either frontal systems or more diffused marginal zones between systems of upper layer circulations [e.g. (Peterson and Stramma, 1991)]. The waters belonging to oligotrophic gyres were distinguished from temperate waters by the presence of Prochlorococcus cyanobacteria (Zubkov et al., 1999a). The waters of the complex system of the equatorial region were geographically identified as extending from the vicinity of the farthest eastward extent of the South American continent in the south to the oceanic waters affected by the proximity of the Mauritanian upwelling close to the West African coast in the north (Figure 1). A good indicator of this latter province was another cyanobacterium, Synechococcus, which reached relatively high numbers above \(10^4\) cells ml\(^{-1}\) in the equatorial region compared with concentrations an order of magnitude lower, at about \(10^3\) cells ml\(^{-1}\), in real oligotrophic regions. Thus, five provinces were distinguished along the transect, namely, the northern temperate waters (NT), the northern Atlantic gyre (NG), the equatorial region (EQ), the southern Atlantic gyre (SG) and the southern temperate waters (ST), as indicated in Figure 6.

Comparison of biogeochemical provinces of the Atlantic Ocean

The relative contributions of each layer to some microbial activities in an integrated 200 m water column are presented in Figure 7. Although the absolute values of parameters were visibly different on the two cruises (as reported above),
Fig. 6. The cumulative layer-integrated data for (a) biomass of heterotrophic bacteria (HB); (b) biomass of heterotrophic nanoplanke (HNP); and production of heterotrophic bacteria measured by simultaneous incorporation of $^3$H-thymidine (Thy, c) and $^{14}$C-leucine (Leu, d) in the three layers along the transect on AMT-3 and AMT-4. Vertical dashed lines indicate the boundaries of the five provinces described in the text.
the averaged structure of the water column typical for each of the provinces was remarkably conservative, as can be seen by comparing left and right columns. The contribution of bacterial stock in each of the three layers was almost equal in both temperate regions. The surface mixed layer L.1 was slightly enriched in heterotrophic nanoplankton. The main amount of both bacteria and nanoplankton was concentrated in the pycnocline layer, L.2, of the northern gyre and in the mixed layer, L.1, of the southern gyre. There was a close to even balance in the equatorial region. A major portion of up to 60% of bacterial production was synthesized in the mixed layer, L.1, in temperate waters and in the southern gyre, while in the equatorial region and the northern gyre, bacteria produced more in the pycnocline layer L.2. The contribution of the deep layer, L.3, to total bacterial production did not exceed 15% in any province and was generally below 10%. Thus, the composition and activity of the microbial community within a province remained rather conservative irrespective of the cruise and perhaps irrespective of the season.

Mean values of biomass concentration and growth rate parameters measured in each of these provinces on both cruises (based on 5–14 stations in each province) are summarized in Figure 8. Generally, the values of bacterial and heterotrophic nanoplankton biomass were similar. The mixed layer, L.1, in temperate waters at both ends of the transect was enriched in heterotrophic bacteria with relatively high concentrations of heterotrophic nanoplankton (Figure 8a). The mixed layer in the equatorial region was also moderately enriched in bacteria and heterotrophic nanoplankton compared with the oligotrophic gyres. The proportions of heterotrophic bacteria and heterotrophic nanoplankton concentrations in both gyres were remarkably consistent on both cruises. The same trends persisted in underlying layers. In the pycnocline layer L.2, most mean values were reduced and the differences between provinces became less pronounced. A high and variable concentration of heterotrophic nanoplankton stood out in the sharply stratified and complex equatorial region. In the deepest layer L.3, all values decreased substantially. This layer was significantly more uniform, but oligotrophic waters still tended to have lower concentrations of heterotrophic bacteria and heterotrophic nanoplankton than mesotrophic temperate waters. The size of the error bars indicates that stocks vary considerably, with greater variation in more productive regions than in oligotrophic regions, and with largest variation in the surface mixed layer whose spatial variability was most evident.

The specific growth rate of heterotrophic bacteria in the surface mixed layer L.1, although relatively variable, was rather similar in all provinces at a level of about 0.1 day⁻¹ (Figure 8b). In the second layer, it decreased slightly in all regions to about 0.07 day⁻¹, except in the equatorial region where it actually increased from 0.9 to 0.12 day⁻¹. In the deepest layer the rates decreased further to 0.01–0.04 day⁻¹. The rates estimated using two precursors were generally similar in all provinces; differences were within standard deviation intervals. Nevertheless, estimates derived from the uptake of leucine were generally higher than those derived from the uptake of thymidine. This difference was prominent in all layers of oligotrophic regions and in deeper layers of other regions. However, the rates
of thymidine-based estimates were somewhat higher than those based on leucine in all layers of southern temperate waters.

Estimation of the balance between production and mortality of natural heterotrophic bacteria

Bacterivory experiments were performed using samples from the mixed layer L.1 only, and at a reduced number of stations, due to the complexity of these
measurements (see Method). These experimental measurements allow the determination of the proportion of bacterial biomass (pulse-chase labelled with either $^3$H or $^{14}$C) which is assimilated by protists. The duration of these experiments was 13 h and for purposes of comparison we calculated these rates as percentages of bacterial standing stock produced or assimilated per day. Nine experiments were conducted on the AMT-3 and 17 experiments on the AMT-4. Assimilation of both labels by heterotrophic nanoplankton was detected in all nine experiments of the first cruise. On the second cruise assimilation of $^3$H could...
not be determined at one station and the assimilation of $^{14}$C was undetectable at four stations in oligotrophic regions.

The actual grazing rate can be calculated by dividing the assimilation of $^{14}$C label by the assimilation efficiency of the $^{14}$C label, which it was possible to estimate in mesotrophic waters to be, on average, $58 \pm 29\%$ ($n = 6$) (Zubkov et al., 1998b). However, we decided to present the original estimates in order to reduce the number of conversion factors involved. To compare the assimilation rates with the daily production of heterotrophic bacteria, the latter was divided by the total biomass of heterotrophic bacteria estimated by flow cytometry and presented graphically as relative bacterial production versus heterotrophic nanoplanckton assimilation (Figure 9). The data for experiments on individual samples are shown in Figure 9a and b, and the mean values for each province in Figure 9c and d. The average relative bacterial production was highest in the northern temperate waters at about 15% day$^{-1}$, compensated by 4% day$^{-1}$ assimilation by heterotrophic nanoplanckton of $^{3}$H label and 13% day$^{-1}$ of $^{14}$C label. In oligotrophic gyres, 6% daily production was balanced with 3–4% assimilation according to $^{3}$H label, and 11–12% production was balanced with 8–12% assimilation according to $^{14}$C label. Similar values were found in the equatorial and southern temperate regions. The proportion of labels from pulse-labelled natural bacteria retained by heterotrophic nanoplanckton was generally less for the $^{3}$H label than for the $^{14}$C label. The lower retention by protists of $^{3}$H label in comparison with $^{14}$C label was observed before for both dual-labelled laboratory cultured and natural bacteria (Zubkov and Sleigh, 1995; Zubkov et al., 1998b). It is clear that when calculated in this way, the assimilation of $^{14}$C label is of the same order as bacterial production. This leads to the conclusion that these processes were in relative balance, although a considerable variance can be observed in the individual estimates scattered in each province (Figure 9a,b) and from the size of the error bars (Figure 9c,d).

Discussion

The layer approach to the investigation of the microbial loop employed in the present study involves certain assumptions that we consider to be justified by the results. Bacterial concentrations measured directly in pooled layer samples were compared with concentrations calculated by integrating data from individual profile samples within these layers. The resulting good agreement ($r^2 = 0.94$, slope $0.95 \pm 0.02$, $n = 67$) confirms that pooled samples adequately characterized the integrated layers. However, it is arguable that standing stocks and physiological activities of micro-organisms are parameters of different types, and that the latter can be affected by mixing the pooled samples much more than the former. In fact, mixing should have little influence on the enzyme measurements in the present case because cells were collected on filters and subsequently lysed very soon after mixing; therefore, there is not much difference in this respect between the process of enzyme assay and counting of fixed picoplanckton cells. Our previous experience showed that uptake of precursors by heterotrophic bacteria at different depths within the top mixed layer, L.1, in oligotrophic waters is consistent [e.g.
The uptake rates in the deep layer, L.3, were low (Figures 4 and 7) and mixing is unlikely to affect them substantially, but perhaps the uptake rates in the pooled intermediate layer, L.2, are most likely to be influenced by mixing. While some caution has to be exercised, we believe that any artifacts generated by mixing are on a far smaller scale than the main latitudinal trends observed, and that the advantage gained by the layer approach outweighs any disadvantage.

The biomass of heterotrophic bacteria was generally somewhat higher in samples collected on AMT-4 than on AMT-3. These differences between the biomasses recorded in the two seasons could not be attributed to the use of two different dyes (TOTO and SYBR Green I) for counting stained bacteria on AMT-3 and AMT-4 because when the same samples were stained in parallel with one or other of the two dyes, the measured bacterial concentrations were identical (Zubkov et al., 1999a). The difference in heterotrophic bacterial biomass between cruises is particularly obvious in the equatorial and southern tropical regions between about 20°N and 20°S, and it is accompanied by similar trends in both the biomass of heterotrophic nanoplankton and the production of heterotrophic bacteria (Figures 3 and 4), and also changes in global temperature.

Fig. 9. Comparison of bacterial growth and grazing. The daily heterotrophic bacterial production (triangles pointing up) and corresponding assimilation of bacterial biomass by heterotrophic nanoplankton (triangles pointing down) measured using $^3$H (a) and $^{14}$C (b) labels, when production and assimilation are expressed as a percentage of the total heterotrophic bacterial biomass in the sample. The open triangles show results from AMT-3 and closed triangles show results from AMT-4. The province mean values are shown as columns for $^3$H (c) and $^{14}$C (d) labels, and error bars indicate the corresponding standard deviation. Vertical dashed lines indicate province boundaries.
distribution (Figure 2). Therefore, the observed difference is likely to be real and, perhaps, reflects global seasonal changes. For example, the temperature of the mixed water layer, even in the southern tropical regions, was about two degrees higher on AMT-4 than on AMT-3 (Figure 2). However, longer term monitoring of the microbial community along the Atlantic Meridional Transect would be necessary to derive more general conclusions.

We employed two precursors for estimating both bacterial production and consumption because the uptake of leucine characterizes the rate of protein synthesis of bacteria, and uptake of thymidine characterizes the rate of synthesis of nucleic acids, DNA in particular [e.g. (Ingraham et al., 1983)]. The former is more an estimate of production of biomass and the latter, of production of bacterial cells. Therefore, in reality, two different aspects of bacterial growth are studied by dual labelling. This distinction is based on studies of bacterial cultures, but incorporation of precursors by natural bacteria can differ significantly from laboratory cultures [e.g. (McDonough et al., 1986; Torreton and Bouvy, 1991)]. Therefore, any conclusions have to be treated with some caution. Normally, the production of biomass and the production of cells might be expected to be in balance in a continuously growing bacterial population. However, comparison of the relative rates of DNA and protein syntheses during the growth of natural assemblages of heterotrophic bacteria in dilution cultures suggests that unbalanced growth may occur and can be observed in mixed assemblages (Chin-Leo and Kirchman, 1990). As in previous studies [e.g. (Chin-Leo and Kirchman, 1988; Zubkov et al., 1999b)], a significant correlation was observed in the present study between production estimated using these two alternative precursors, leucine and thymidine, in the upper two layers, L.1 and L.2, on both AMT-3 and AMT-4 cruises (Figure 5a). Therefore, in spite of the compositional complexity of the bacterial community, its communal (integral) growth gave the appearance of remarkably balanced growth in surface waters across the whole Atlantic Ocean. Additionally, the same, general, empirical conversion factor, derived from a set of dilution cultures, was used for estimating production at an oceanic scale. However, the regularly observed higher estimates derived from leucine uptake rates compared with thymidine uptake rates, particularly in oligotrophic gyres, suggest that these bacterial communities are growing somewhat differently from communities inhabiting more productive mesotrophic waters that are more likely to be modelled by the dilution culture experiments used for determining the conversion factors. Here, we have applied conversion factors for converting precursor uptake rates into bacterial production which have been derived from the monitoring of actively-growing dilution cultures. The uncoupled specific growth rates revealed by the application of these conversion factors to regions where nutrient concentrations are constantly low may be the result of genuine imbalance or may reflect the use of inappropriate conversion factors in this situation.

One of the interesting features observed in this study was a conservative distribution of measured parameters along the transect that allowed the implementation of a province approach (Figure 6). These latitudinal differences in microbial biomasses and rates agree with previous observations (Buck et al., 1988; Buck et al., 1993).
As mentioned above, seasonality did affect the microbial communities of equatorial and temperate waters and, to a lesser extent, of the oligotrophic gyres. Nevertheless, the relative microbial characteristics of the water column remained conservative (Figure 7). The usefulness of a province concept for studies at an oceanic scale lies in the ability to characterize large water areas by averaged and thus, more precise values of heterotrophic bacterial and heterotrophic nanoplankton stocks, heterotrophic bacterial production and heterotrophic nanoplankton grazing rates that can be readily employed in modelling. It is clear that in biological oceanography, the ability to achieve adequate sampling coverage of vast water areas is restrained by laborious methodology and therefore, it would be more practical to identify the extent of a certain province by means of rapid survey, space- or ship-based, and then to representatively sample its waters to obtain mean values of key parameters that characterize the dynamics of, for example, the microbial loop. The comparison of such values in five provinces revealed much more similar specific growth rates of bacteria populating the surface mixed layer than might be expected and somewhat less striking similarities of HB and HNP stocks in the aphotic third layer (Figure 8).

The specific growth rate of heterotrophic bacteria, calculated as the natural logarithm of the relative increase of total biomass of heterotrophic bacteria, varied from 0.06 to 0.12 day\(^{-1}\) in the mixed layer L.1 (Figure 8b), representing a doubling time of about 5.5–14 days. These values appear similar to, or at the lower end of the range of values reported for various regions of the Atlantic Ocean (Table I). The picture of the dynamics of heterotrophic bacterial populations emerging from this study can be linked closely with results obtained on a previous cruise along the 20\(^\circ\)W meridian between 60\(^\circ\)N and 36\(^\circ\)N during June and July 1996 (Zubkov et al., 1999b). In that summer study, the specific growth rate of heterotrophic bacteria in the top mixed layer decreased from about 0.15–0.2 day\(^{-1}\) in temperate waters to 0.1–0.14 day\(^{-1}\) in oligotrophic waters. The equality of values of the heterotrophic bacterial and heterotrophic nanoplankton biomasses in oligotrophic waters is comparable with data reported for the Sargasso Sea [e.g. (Caron et al., 1995, 1999)].

There are very few estimates of rates of bacterivory in oligotrophic waters (Thingstad et al., 1996; Caron et al., 1999) because of methodological difficulties involved in these measurements. Based on our results, we can conclude that heterotrophic nanoplankton can tightly control the growth of metabolically-active heterotrophic bacteria and can be responsible for consumption of the total daily production of heterotrophic bacteria in all studied provinces of the Atlantic Ocean. This agrees with the conclusion made in other studies of bacterivory in similar or more productive regions [e.g. (Wikner et al., 1986; Hagström et al., 1988; Sherr et al., 1989; Caron et al., 1999)]. In the case of tight predator pressure, it could be spatial and temporal (seasonal) patchiness that causes fluctuations of standing stocks of bacteria and protists (see scatter of measurements in Figure 9). Additionally, we found that different classes of bacterial macromolecules are digested by protozoa with different efficiency. Radio-labels from bacterial nucleic acids were largely released by wild protozoan predators, but to a lesser extent...
than in culture, and bacterial proteins were assimilated by the protozoans with high efficiency.

However, there are certain potential problems associated with balancing bacterial production and mortality (Zubkov et al., 1998b; Caron et al., 1999). In the present study, the assimilation of bacterial protein labelled with $^{14}$C was almost equivalent to the bacterial production (Figure 9). The explanation why measured production was found to equal protozoan assimilation is most likely that some of the counted bacteria were not labelled because not all of them were metabolically active. The relative production will be underestimated if inactive bacteria are present because the isotope uptake by active bacteria is considered to represent production by all bacteria (active + inactive), while bacterivory is estimated as a percentage of pulse-chase labelled and thence, only active bacteria. The question of the proportion of active, inactive and dead bacteria recently attracted considerable attention [e.g. (Gasol et al., 1995; Jepras et al., 1995; Zweifel and Hagström, 1995; del Giorgio et al., 1997)]. To determine which cells are metabolically active, it is usual to use fluorescent dyes, either directly or as a result of enzymatic reaction in or on the surface of the cells. In our case, this would be almost impossible because even in eutrophic water, about 10 h of

Table I. Comparison of ranges of heterotrophic bacterial biomasses, bacterial growth rates and heterotrophic nanoplanckton biomasses in the surface mixed layer for several recent studies in the Atlantic Ocean

<table>
<thead>
<tr>
<th>Region</th>
<th>Season</th>
<th>HB biomass mg C m$^{-3}$</th>
<th>Growth rate day$^{-1}$</th>
<th>HNP biomass mg C m$^{-3}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Atlantic</td>
<td>Spring</td>
<td>20–60 (20 fg C cell$^{-1}$)</td>
<td>0.1–0.3$^{T,L}$</td>
<td>–</td>
<td>(Ducklow et al., 1993)</td>
</tr>
<tr>
<td>47ºN, 20ºW</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>North Atlantic</td>
<td>Spring</td>
<td>30–40 (20 fg C cell$^{-1}$)</td>
<td>0.08–0.25$^{T,L}$</td>
<td>–</td>
<td>(Li et al., 1993)</td>
</tr>
<tr>
<td>40–45ºN, 41–47ºW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gulf Stream</td>
<td>Autumn</td>
<td>6–29 (350 fg C µm$^{-3}$)</td>
<td>0.02–0.39$^{T}$</td>
<td>–</td>
<td>(Børshheim, 1990)</td>
</tr>
<tr>
<td>32–38ºN, 72–77ºW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sargasso Sea</td>
<td>Spring,</td>
<td>2–18 (15 fg C cell$^{-1}$)</td>
<td>0.17–0.56$^{T}$</td>
<td>1–14</td>
<td>(Caron et al., 1999)</td>
</tr>
<tr>
<td>summer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Atlantic</td>
<td>Summer</td>
<td>4–18 (14 fg C cell$^{-1}$)</td>
<td>0.05–0.2$^{T,L}$</td>
<td>2–8</td>
<td>(Zubkov et al., 1999b)</td>
</tr>
<tr>
<td>37–59ºN, 20ºW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Atlantic</td>
<td>Spring,</td>
<td>2–10 (7 fg C cell$^{-1}$)</td>
<td>0.04–0.17$^{T,L}$</td>
<td>3–12</td>
<td>This study</td>
</tr>
<tr>
<td>38–48ºN, 20ºW</td>
<td>autumn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Gyre</td>
<td>Spring,</td>
<td>1–2 (7 fg C cell$^{-1}$)</td>
<td>0.04–0.17$^{T,L}$</td>
<td>1–3</td>
<td>This study</td>
</tr>
<tr>
<td>23–38ºN, 20ºW</td>
<td>autumn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equatorial Region</td>
<td>Spring,</td>
<td>1–6 (7 fg C cell$^{-1}$)</td>
<td>0.04–0.15$^{T,L}$</td>
<td>1–7</td>
<td>This study</td>
</tr>
<tr>
<td>23ºN–8ºS, 20–29ºW</td>
<td>autumn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern Gyre</td>
<td>Spring,</td>
<td>1–2.5 (7 fg C cell$^{-1}$)</td>
<td>0.03–0.16$^{T,L}$</td>
<td>1–3</td>
<td>This study</td>
</tr>
<tr>
<td>8–33ºS, 29–37ºW</td>
<td>autumn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Atlantic</td>
<td>Spring,</td>
<td>1–7 (7 fg C cell$^{-1}$)</td>
<td>0.04–0.16$^{T,L}$</td>
<td>1–12</td>
<td>This study</td>
</tr>
<tr>
<td>33–48ºS, 37–55ºW</td>
<td>autumn</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Carbon conversion factors, either per cell or per unit volume (in cases where bacterial volumes were estimated by microscopy), used to calculate biomass of heterotrophic bacteria are presented in parentheses.

$^{T}$ Thymidine was used as a precursor to estimate bacterial production.

$^{T,L}$ Thymidine and leucine were used as precursors to estimate bacterial production.
incubation is required to develop sufficient respiratory-dependent staining with CTC for detection by flow cytometry (Gasol et al., 1995); furthermore, some metabolically-active bacteria may not use this form of metabolism. Therefore, the estimation of the proportion of inactive bacteria in oligotrophic oceanic waters remains a challenge.

If the reported dataset is analysed as a whole, there are some interesting general questions to be considered in relation to carbon flux through the microbial loop. Standing stocks of heterotrophic bacteria were generally similar to the estimated standing stock of heterotrophic nanoplankton (Figures 3 and 8a). A positive relation between bacterial biomass and production was evident in the top mixed layer L.1 (Figure 5b). Daily production of heterotrophic bacteria in this surface mixed layer, which was 10–15% of the standing stock of heterotrophic bacteria depending on the province, was consequently about 10% of the heterotrophic nanoplankton biomass and, according to estimated bacterivory, heterotrophic nanoplankton can consume it all with high efficiency. Thus, heterotrophic nanoplankton, whose efficient digestion enables the assimilation of up to 10% of their biomass daily from the consumed heterotrophic bacteria, have a potential (in the absence of severe grazing pressure on heterotrophic nanoplankton) to grow at the same rate or faster than heterotrophic bacteria. It should also be remembered that phototrophic cyanobacteria, the biomass and production of which surpass the biomass and production of heterotrophic bacteria [e.g. Zubkov et al., 1999a)], are another source of prey for heterotrophic nanoplankton. If we assume that the estimates are credible, heterotrophic nanoplankton may grow at a rate of 0.1 day⁻¹ and should be under very tight grazing pressure from microzooplankton. This is most likely to be the case because we observe only weak, or no correlation between standing stocks of bacteria and heterotrophic nanoplankton (Figure 5d), although there was a possible relationship between heterotrophic nanoplankton biomass and production of bacterial prey (Figure 5c). Therefore, there should be alternative control of the heterotrophic nanoplankton population by a top predator.

However, our conclusions are dependent on certain methodological assumptions and conversion factors (e.g. Table I which reveals variation in estimates of bacterial biomass associated with the use of different conversion factors). Also, an alternative explanation cannot be discarded that the biomass of heterotrophic nanoplankton can be underestimated or that bacterial biomass can be overestimated. Microscopic counting of fixed cells on filters is not very accurate, even for less complicated groups like bacteria [e.g. (Karl, 1994)], and must be approximate for heterotrophic nanoplankton. Another uncertainty remains unresolved and that is the C:volume conversion factor used to estimate biomass of bacterial cells. This question has received considerable attention during the last two decades [(Nagata and Watanabe, 1990; Christian and Karl, 1994; Fagerbakke et al., 1996) and references therein]. While a lot of experimental work has been done, a uniform conversion factor has not been agreed; probably, a single factor would not be appropriate and it would be more accurate to measure carbon content directly if it is methodologically possible. Nonetheless, it should be possible to trace carbon flow through the different
groups of the plankton community, and a ‘single currency’ of some unit such as C biomass is needed. Estimates of the C:volume conversion factor for heterotrophic bacteria span almost an order of magnitude [0.05–0.38 pg C µm−3 (Christian and Karl, 1994)], and we have used a factor of 0.12 pg C µm−3, which is close to the direct estimates made with X-ray microanalysis (Fagerbakke et al., 1996) and the same as a conversion factor employed in recent studies in the Sargasso Sea (Carlson et al., 1996). At the estimated values of heterotrophic nanoplanckton biomass, they could control the populations of both heterotrophic and phototrophic bacteria, cropping all production of the former and, perhaps, of the latter as well.

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Bacterial dynamics in Atlantic waters


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