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Bottom-up and top-down control of bacterioplankton from eutrophic to oligotrophic sites in the tropical northeastern Atlantic Ocean

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Abstract—Bacterioplankton biomass and production were determined over the whole water column of a eutrophic, a mesotrophic and an oligotrophic site (2300, 3200 and 4500 m deep) in the tropical NE Atlantic Ocean during a EUMELI cruise in May–June 1992. This resulted in an exceptionally large amplitude of data, with abundance, biomass and production ranging from 1.2×10^7 to 3.9×10^9 cell l^{-1}, 1.2×10^{-7} gC l^{-1} to 4.7×10^{-5} gC l^{-1} and 2.0×10^{-11} gC l^{-1} h^{-1} to 9.6×10^{-7} gC l^{-1} h^{-1}, respectively. These data were analyzed in order to determine whether bacterioplankton was controlled by bottom-up or top-down processes. Regressions of log-transformed bacterioplankton biomass versus log-transformed production were tested at the different sites and in different layers. Slopes of 0.40–0.55 suggest that bacterioplankton is moderately controlled by bottom-up processes increasing from the eutrophic coastal site to the open ocean oligotrophic site and from surface layers (< 250 m) to deep waters. These conclusions are in agreement with other indices of bottom-up control like cell size, doubling times of the biomass, per cent of active cells, and relationships of bacterial biomass versus phytoplanktonic biomass and production. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Determining the type of control exerted on planktonic populations over different time and space scales is essential in order to understand oceanic fluxes. Regarding bacterioplankton, one has to know whether population size is limited by food supply (labile carbon, nitrogen, phosphorus) or other factors limiting bacterial growth (temperature, pressure) or by bacterivores and other loss mechanisms (advection, sedimentation, non-predatory mortality). In other words, what is the relative importance of the opposing "bottom-up" or "top-down" regulatory mechanisms? Various approaches, including comparative and experimental studies (Pace and Cole, 1994), have been employed to address this central question in aquatic microbial ecology.

Bottom-up control of bacterioplankton biomass is supported by the results from Cole *et al.* (1988), Billen *et al.* (1990), Simon *et al.* (1992), Kirchman *et al.* (1993) and Shiah and Ducklow (1994) using different approaches on marine and freshwater systems of very different trophic status. Top-down control is indicated by several kinds of experiments and field observations by Davis and Sieburth (1984), Ducklow and Hill (1985), Rassoulzadegan and Sheldon (1986), Painting *et al.* (1989), Kuparinen and Bjornsen (1992) and Psenner and Sommaruga (1992).

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The control of bacterioplanktonic biomass cannot evidently be dissociated from the observational window (Ducklow, 1984) and may vary with the time or space scale considered (Ducklow, 1992). In fact, much of the evidence used in support of bottom-up or top-down hypotheses in marine waters have been obtained from estuarine or coastal systems and surface waters, and there are still few data for the open ocean, especially throughout the water column.

Here, we examine the processes regulating bacterioplankton biomass in the whole water column of three sites of the Atlantic Ocean presenting contrasting trophic status in a single climate zone. These processes are examined primarily by a comparative approach developed on bacterioplankton by Billen *et al.* (1990) and are compared with other indices of the nature of control exerted on bacterioplankton biomass.

SITES AND METHODS

Data were collected in May and June 1992, north of Cape Verde, West Africa, at latitudes 20–21°N during the EUMELI cruise IV. Background oceanographic information about this cruise is presented in Jacques (1993) and Morel (1994). Three sites of different trophic status were investigated. The eutrophic site (18°W, 20.5°N), about 90 miles from the Mauritanian coast, is enriched by an upwelling current. The site at 21°W, 18.30°N, is located in the frontal zone between the Central North Atlantic and Central South Atlantic water masses. It is considered to be mesotrophic. The most oligotrophic site is located at 31°W, 21°N, in the North Equatorial Current.

Surface temperature was 23°C at both the oligotrophic and mesotrophic sites, and 18.5°C at the eutrophic site. It was 21, 17 and 18.5°C at the bottom of the euphotic layer of these three sites. The euphotic layer was about 150, 100 and 50 m deep at the oligotrophic, mesotrophic and eutrophic sites, and the mixed layer was about 35, 40 and 50 m thick. Within the euphotic layer of the oligotrophic, mesotrophic and eutrophic sites, chlorophyll *a* concentrations were 0.22, 0.47 and 1.35 mg m^{-3} on average, and primary production was 300, 900 and 1300 mgC m^{-2} day^{-1}.

Water samples were collected with acid-washed Go-Flo bottles from the surface to 4000, 3000 and 2200 m at the oligotrophic, mesotrophic and eutrophic site. Bacterial enumerations were performed on samples filtered on 0.2 μm Nuclepore membranes, after staining with DAPI (Porter and Feig, 1980). An epifluorescence microscope suspended on a vibration isolation table (Technical Manufacturing Corporation, Peabody, MA) allowed bacterial enumeration onboard less than 2 h after sampling or, if delayed, on frozen preparations (−20°C). More than 400 cells on at least 20 fields were enumerated. Bacterial volumes were computed from bacterial dimensions estimated on enlarged photographs (Lee and Fuhrman, 1987). Bacterial carbon was deduced from bacterial volumes following the allometric model of Simon and Azam (1989) modified by Norland (1993).

Active bacteria were defined as cells able to reduce 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) in formazan (Zimmerman *et al.*, 1978) using a modification of the original method (Dufour and Colon, 1992). Duplicate 20–100 ml subsamples were amended with INT (0.02% final concentration) and incubated in the dark at *in situ* temperature ± 1°C for 0.5 h in surface waters to 3 h in deep waters. Incubation was terminated by addition of buffered formalin (2% final concentration). Bacteria were then stained with DAPI, collected onto 0.1 μm pore size cellulose nitrate membranes and observed under epifluorescence and transmission light as described in Dufour and Colon

(1992). INT reducing bacteria, presenting dense intracellular deposits of INT-formazan, were enumerated under the microscope. At least 100 INT reducing cells were enumerated on at least 20 fields.

Bacterial production was estimated from TdR incorporation (Fuhrman and Azam, 1982). On 500 m deep samples and at upper levels, TdR incorporation was assayed by amending duplicate 20–100 ml subsamples with 20 nM [methyl-^{3}H]thymidine (final concentration, Amersham, 1.74 TBq mmol^{-1}). After 0.5–3 h incubation with the label at *in situ* temperature ($\pm$1°C), the duplicates were chilled in a 2°C water bath for 10 min. Samples were then filtered onto 0.2 µm Nuclepore polycarbonate membranes and rinsed with 5 ml of 0.2 µm filtered seawater. The vacuum was disconnected, and filters received 15 ml ice-cold 5% TCA. After 15 min, vacuum was reapplied and the membranes were rinsed three times with 5 ml of ice-cold 5% TCA. Labeled DNA was extracted enzymatically following a modification (Torréton and Bouvy, 1991) of the Wicks and Robarts (1987) procedure. Radioactivity was determined after quench correction with external standards. Incorporation was calculated after subtracting a zero time blank. Linearity of incorporation over the periods of incubation was verified regularly, and TdR incorporation rates always saturated at less than 20 nM TdR (checked twice at every site, data not shown). Below 500 m, TdR incorporation was performed by incubating single 1-l sub-samples with 5 nM [methyl-^{3}H]thymidine at *in situ* temperature ($\pm$1°C) and atmospheric pressure. A total of five 160 ml subsamples were retrieved from the incubation bottle every 6 h from 0 to 24 h. The incorporation rate into cold-TCA precipitate was always linear over the 24 h incubation ($r^2 > 0.87$). DNA was extracted enzymatically from an additional 160 ml subsample at the end of the incubation period (24 h). We made no measurement below 1000 m at the oligotrophic site. The median value from the literature (2×10^{18} cells mol^{-1}, Ducklow and Carlson, 1992) was used to convert [^{3}H]-thymidine incorporation into bacterial production.

Chlorophyll *a* and primary production data were measured according to Neveux and Panouse (1987) and Dandonneau and Le Bouteiller (1992) and were kindly provided by J. Neveux and Y. Dandonneau.

RESULTS

Ranges of bacterial biomass and production

Bacterial abundance ranged from 1.2×10^7 to 3.9×10^9 cell l^{-1} over the three sites. Biomass covered more than two orders of magnitude, ranging from 1.2×10^{-7} gC l^{-1} to 4.7×10^{-5} gC l^{-1}, whereas production covered nearly five orders of magnitude from 2.0×10^{-11} gC l^{-1} h^{-1} to 9.6×10^{-7} gC l^{-1} h^{-1}. Bacterial biomass and production data used in this study are summarized in Fig. 1.

Billen's approach

In order to examine the regulation of bacterial population by resource availability, data were analyzed according to the approach suggested by Billen *et al.* (1990). Their basic assumption is that, near steady-state conditions, substrate supply rate should be balanced by substrate use. Accordingly, admitting a nearly constant growth yield, bacterial

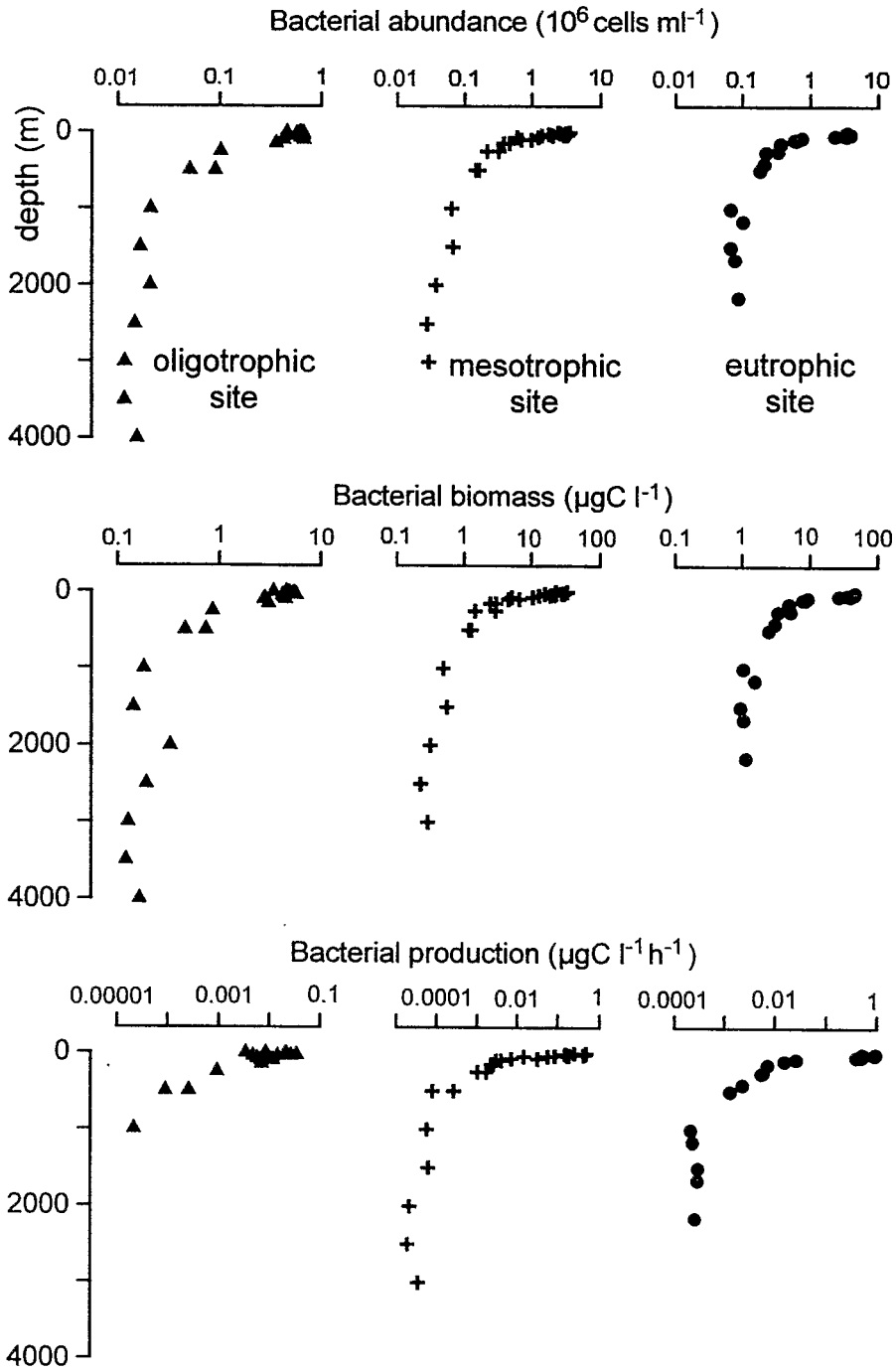

Fig. 1. Depth distribution of bacterioplanktonic abundance, biomass and productivity at the eutrophic (•; 24–28 May 1992), mesotrophic (+ May 31–June 4 1992) and oligotrophic sites (▲; 5–6 June 1992).

production rates can be used as estimates for substrate supply in a bottom-up regression of bacterial biomass versus bacterial production.

Log–log linear regression of bacterial biomass on bacterial production for our pooled data is highly significant ($r^2 = 0.96$, $p < 0.001$, $n = 68$, Table 1 and Fig. 2). The slope of 0.48 suggests, following Billen *et al.* (1990), that bacterial biomass is moderately controlled by resource supply.

The variations of log (bacterial production) explain 96% of the variations of log (bacterial biomass). But biomass is a composite parameter of cell abundance and carbon content per

Table 1. Statistics for log–log linear regressions of bacterial biomass on bacterial production

Source	CCF*	TCF†	*N*	Slope ± SE	*Y*-int ± SE	*R*(BP)‡	*R*(Sp)§	Reference
Pooled data	Norland	2	68	0.48±0.01	1.68±0.03	0.98	0.96***	This study
Pooled data	S and A	2	68	0.47±0.01	1.72±0.03	0.98	0.96***	This study
Pooled data	L and F	2	68	0.47±0.01	1.84±0.03	0.98	0.95***	This study
Oligotrophic ≥250 m	Norland.S	2	17	0.40±0.08	1.40±0.16	0.78	0.73**	This study
Mesotrophic ≥250 m	Norland.S	2	18	0.42±0.03	1.66±0.06	0.96	0.95***	This study
Eutrophic ≥250 m	Norland.S	2	16	0.48±0.02	1.74±0.02	0.99	0.91***	This study
Pooled data <250 m	Norland.S	2	17	0.55±0.06	1.99±0226	0.93	0.81***	This study
Drinking to river water	S and A	0.5–5	288	0.7	1.67	0.91		Billen *et al.* (1990)
Chesapeake bay + open oc.	L and F	1.2–4	2519	0.46	1.82	0.88		Ducklow (1992)
Open ocean	L and F	1.2–4	1296	0.28	1.50	0.57		Ducklow (1992)

*CCF = Bacterial cell carbon: conversion factor. S and A, according to the carbon to volume relationship of Simon and Azam (1989): $C = 0.09V^{0.60}$, with C in pg μm^{-3} and V in μm^3. Norland according to the recalculation of the Simon and Azam relationship by Norland (1993): $C = 0.12V^{0.72}$. L and F, according Lee and Fuhrman (1987): 20 fgC $cell^{-1}$.

†TCF = Thymidine conversion factor in 10^{18} cells mol^{-1}.

‡*R*(BP) = Bravey Pearson's correlation coefficient.

§*R*(Sp) = Spearman's rank correlation coefficient, ** $p < 0.01$; *** $p < 0.001$.

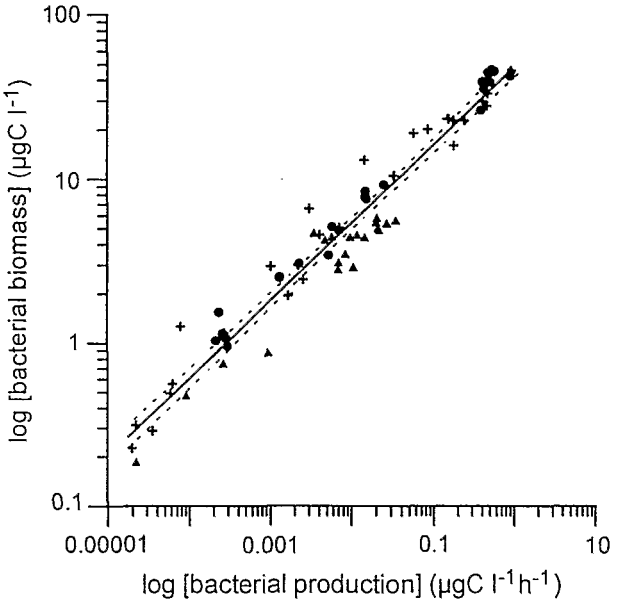

Fig. 2. Log–log linear regression of bacterial biomass on bacterial production at the eutrophic (•), mesotrophic (+) and oligotrophic sites (▲). Dashed lines represent 95% confidence interval for regression slope.

*Table 2. Some parameters indicative of the bottom-up control of bacterial biomass. Turnover time of biomass, cellular volume, and per cent of active cells mean ± standard deviation (*n *data) in the euphotic zones of the three sites*

Site	Turnover time (d)	Cell vol. (μm^3)	% Active cells
Oligotrophic	18 ± 12 (16)	0.022 ± 0.004 (8)	6.1 ± 2.4 (6)
Mesotrophic	19 ± 24 (14)	0.027 ± 0.005 (7)	4.0 ± 1.6 (5)
Eutrophic	3.2 ± 0.7 (10)	0.042 ± 0.005 (5)	5.5 ± 0.6 (4)

cell, and these parameters may be regulated by different factors (Psenner and Sommaruga, 1992). In some environments, changes in the biovolumes may be responsible for a significant part of the change of bacterial biomass (Billen *et al.*, 1990). We examined how these two independent parameters are related to production. Log (bacterial production) explains 94% of the variations of log (bacterial abundance), whereas it explains only 1.5% of the variations of log (carbon per cell). This difference is likely due to the range of abundance, which covers more than two orders of magnitude, compared to the range of carbon content per cell, which varies only from 6.4 to 15.6×10^{-15} gC cell^{-1}. Clearly, in the sites investigated during this study, bacterial volumes are not responsible for the major increase of bacterial biomass along the production gradient.

Therefore, the choice of carbon conversion factors should have a very limited influence on the regression mentioned above. The Norland (1993) volume dependent relationship was replaced by that originally published by Simon and Azam (1989). We also tested the relationships using 20 fgC cell^{-1}, estimated by Lee and Fuhrman (1987) for cell volumes less than 0.07 μm^3 (this is the case in our study, see Table 2). Changes in the regression were indeed insignificant using these different factors (Table 1). We used a constant value of 2×10^{18} cells mol^{-1} of thymidine incorporated (see Sites and Methods), but the use of other values can change neither the regression slopes nor the correlation coefficients mentioned in

Table 1. At least on a global scale, the choice of conversion factors does not change the conclusion of the existence of a moderate bottom-up control.

We also tested whether the strong correlation observed between biomass and production was due to the amplitude of the variations in the data used. It seems plausible that when the ultra oligotrophic waters of the deep ocean are compared with eutrophic upwelled waters, a significant correlation between the bacterial biomass and production would be observed. Significant relationships were also obtained in most cases at the relatively large scale of oceanic sub-basins (Ducklow, 1992). However, determining the bottom-up or top-down nature of bacterial control should be more meaningful at smaller scales.

We therefore analyzed, separately, our data according to the layers deeper than 250 m and between surface and 250 m for oligotrophic, mesotrophic and eutrophic sites (Table 1). It is noticeable that the log–log linear regressions are close for all the data sets. Significant relationships between bacterial biomass and production are observed in all cases. Correlation coefficients from 0.78 to 0.99, with regression slopes from 0.40 to 0.55, suggest that bacterial biomass is moderately controlled by bottom-up forces.

Regression slopes less than 1 suggest that bacterial response (expressed by biomass) to increasing resource availability (expressed by production) is strongly attenuated. This should substantiate a decreasing pressure of bottom-up processes from the oligotrophic open ocean to the eutrophic coastal ocean and from deep waters to surface waters. This may be due to the increasing effect of adverse top-down processes as discussed later. Other independent results may help to evaluate the level of bottom-up or top-down control in the different situations we encountered.

Cell sizes

It is well known that bacteria increase in size in response to an increase in substrate availability (Roszak and Colwell, 1987; Berman *et al.*, 1994). In the oligotrophic ocean and even in mesotrophic waters, the average bacterial size is small (*ca* 0.05–0.07 μm^3, Simon *et al.*, 1992). Pedrós-Alió and Brock (1982) reported larger cells in eutrophic than in oligotrophic lakes. We estimated mean cell volumes of 0.042, 0.027 and 0.022 μm^3 in euphotic zones at the eutrophic, mesotrophic and oligotrophic sites, respectively (Table 2). No significant size differences were detected between depths (data not shown). Such small sizes should be indicative of resource limited cells.

Turnover times of bacterial biomass

Turnover times of bacterial biomass are shown in Fig. 3. They increase from some days in the surface waters (3 at the eutrophic site, 18 at the oligotrophic site) to several months in the deep-sea layers. It has been shown that populations limited by resources have low biomass renewal rates (Fuhrman *et al.*, 1989; Cho and Azam, 1990). The observed biomass turnover times are indicative of the importance of bottom-up controls on the bacteria that increase from the coast towards the central Atlantic and from the surface to deep waters.

Proportions of active bacteria

The proportion of active bacteria, detected by the INT reduction method, ranges between 4 and 6% in the euphotic zone with no significant differences between sites (Table 2). Active

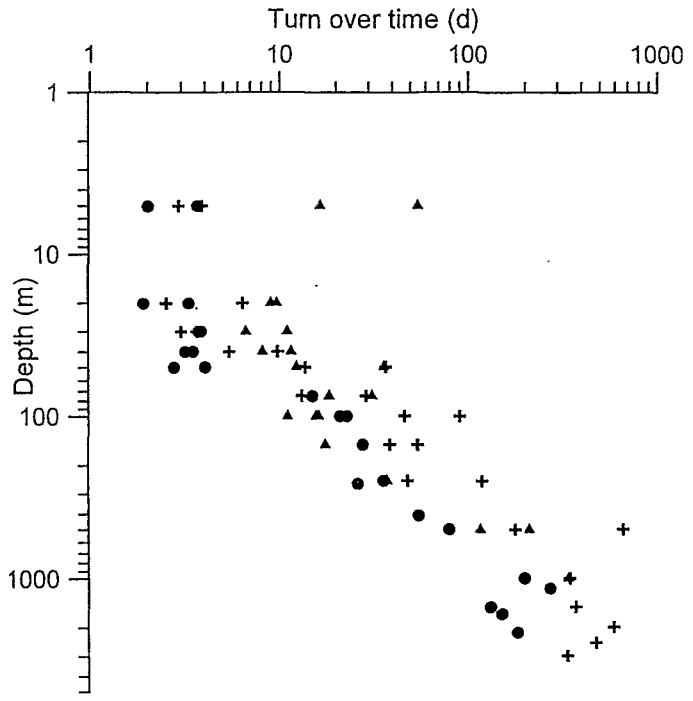

Fig. 3. Turnover time of bacterioplanktonic biomass (biomass/production) at the eutrophic (•), mesotrophic (+) and oligotrophic sites (▲).

cells, enumerated occasionally in the aphotic zone, represent less than 2% of total abundance. Such low percentages suggest a resource limitation in opposition to the high percentages encountered under strong predatory control (Dufour *et al.*, 1990).

Relations between bacterial biomass and phytoplankton

Bottom-up control can also be inferred from correlations between the biomass of nutrient consumers (dependent variable) and the biomass or production of nutrient producers (independent variables; Cole *et al.*, 1988; Simon *et al.*, 1992). Correlations of bacterial biomass versus chlorophyll *a* and primary production at the three sites separately and on pooled data are significant seven times out of eight (Tables 3 and 4). Positive correlations suggest that bacterial biomass is somehow controlled by resources derived from phytoplankton. If phytoplankton is also controlled by resources, bacteria should respond to increases in nutrients used by primary producers. Such an indirect response of bacteria, modulated by the phytoplanktonic response, has been observed in microcosms by Hobbie and Cole (1984) and Bjornsen *et al.* (1988).

The correlation between bacterial biomass and chlorophyll *a* is not significant at the oligotrophic site (Table 3). This may be due to a vertical shift between the subsurface maximum of bacteria and the 100 m deep maximum of chlorophyll *a*. This deep maximum of chlorophyll probably overestimates phytoplanktonic biomass, as cells have a higher chlorophyll content at these low light levels. This is in agreement with the absence of a deep maximum of primary production and particulate carbon, both greater between 20 and 40 m than at around 100 m (data not shown). Therefore, the use of a regression fit between bacterial biomass and chlorophyll, as an index of dependency of bacterial biomass on phytoplanktonic biomass, should be used with caution in oligotrophic water columns.

Table 3. Statistics for log–log linear regressions of bacterial biomass on chlorophyll a in the euphotic zone

Site	*n*	Slope ± SE	*Y*-int ± SE	*R*(BP)†	*R*(Sp)‡
Oligotrophic	16	0.03 ± 0.14	0.67 ± 0.38	0.06	-0.08^{NS}
Mesotrophic	17	0.43 ± 0.06	2.67 ± 0.18	0.89	0.76**
Eutrophic	13	0.39 ± 0.06	3.25 ± 0.18	0.88	0.95***
Pooled data	46	0.45 ± 0.07	2.57 ± 0.21	0.68	0.69***

†*R*(BP) = Bravey Pearson's correlation coefficient.
‡*R*(Sp) = Spearman's rank correlation coefficient.
NS for non-significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 4. Statistics for log–log linear regressions of bacterial biomass on primary production in the euphotic zone

Site	*n*	Slope ± SE	*Y*-int ± SE	*R*(BP)†	*R*(Sp)‡
Oligotrophic	12	0.16 ± 0.04	1.46 ± 0.05	0.81	0.68*
Mesotrophic	13	0.23 ± 0.03	2.74 ± 0.07	0.93	0.87**
Eutrophic	10	0.04 ± 0.01	3.79 ± 0.04	0.78	0.85**
Pooled data	35	0.16 ± 0.07	2.60 ± 0.17	0.37	0.48**

†*R*(BP), ‡*R*(Sp), * and ** as for Table 3.

The slopes of the three significant log–log regressions of bacterial biomass on chlorophyll *a* (Table 3) and of the four regressions of bacterial biomass on primary production (Table 4) are much lower than 1. This suggests that bacterial response to increased resource availability derived from phytoplankton tends to be attenuated over the range of our data.

Using the Norland (1993) relationship for bacterial carbon and a C:chl *a* ratio of 50 (Redalje, 1983), the fitted relationship between bacterial and phytoplanktonic carbon using all the data from the euphotic zone is: Bacterial C (mg m^{-3}) = 0.47 phytoplankton C (mg m^{-3}) + 5.84, $r^2 = 0.63$, $n = 46$. This implies that bacterial carbon exceeds phytoplanktonic carbon when it is less than 11 mg m^{-3} (chl $a < 0.22$ mg m^{-3}). This is in close agreement with previous studies (Cole *et al.*, 1988; Fuhrman *et al.*, 1989; Cho and Azam, 1990 and Simon *et al.*, 1992) showing that bacterial biomass exceeds phytoplankton biomass in the most oligotrophic marine and lacustrine waters.

Relative importance of bottom-up control according to sites and depths

The doubling time of bacterial biomass and probably the cell size (Table 2) are indicative of an increasing bottom-up control from the Mauritanian coast towards the open ocean. The biomass turnover time (Fig. 3), the proportion of active cells (Table 2 and text), and the coefficient of regression of bacterial biomass versus bacterial production (Table 1), all suggest a moderate bottom-up control that increases with depth. Moreover, the log–log regression slopes of bacterial biomass versus bacterial production (Table 1), chlorophyll *a* (Table 3) and primary production (Table 4), all lower than 1, indicate that bottom-up

control decreases with increasing resources. These independent parameters are in agreement and suggest a bottom-up control of bacterioplankton biomass increasing from the coastal ocean to the open ocean and from surface waters to deep waters.

DISCUSSION

Regressions of bacterial biomass versus bacterial production

Other studies have used Billen's approach and considered the regression slope of bacterial biomass on bacterial production as an index of bottom-up control of the bacterial biomass (Table 1). Ducklow (1992) reported a slope of 0.47, similar to ours, on pooled data from Chesapeake Bay and open ocean locations. The open ocean data alone yielded a slope of only 0.28, suggesting that bottom-up control operates only weakly in the open ocean. Billen *et al.* (1990) reported a slope of 0.7, higher than others, mostly because they included data from the Scheldt Estuary and Meuse River, where bacterial biovolume increased sharply at higher production rates.

We are aware that the conclusions drawn from the regression of biomass on production are quite dependent upon the validity of some implicit assumptions. In Billen's approach, bacterial production is used as an estimate of substrate use (equivalent to substrate supply near steady-state). However bacterial production and substrate use are not proportional over a wide range of trophic situations. Both theory and experimental results suggest that growth yield decreases with decreasing growth rates (Middelboe *et al.*, 1992). Therefore, regression of biomass versus substrate consumption would present greater slopes than those reported in this study for biomass versus production. The limited data presently available in the literature about the evolution of growth yield related to growth rate prevents its quantification and therefore the evaluation of substrate consumption. However, this growth yield decrease clearly reinforces the evidence for bottom-up limitations at the observed scales.

Decompression effects

Production and biomass are measured at atmospheric pressure whatever the depth of sampling. The decompression effect on bacterial total abundance and cell size has not yet been studied to our knowledge. If a decompression shock cannot be excluded, it is supposed to have a greater effect on activity than on biomass. Bianchi and Garcin (1993) observed that the decompression of deep samples invariably reduced the activity of bacteria. However, reviewing previous work, they noted opposite effects. They suggested that the decompression of deep samples reduces the activity of autochtonous bacteria but increases the activity of bacteria that have settled from the surface. Moreover, at present it is impossible to assess the depth at which decompression may alter productivity measurements and to estimate, with reasonable confidence, the importance of the bias created by decompression. If we admit that decompression leads to an underestimation of bacterial production and that this bias increases with the depth of sampling, the true regression slope of bacterial biomass versus *in situ* production should be higher than the one we report. Therefore, our conclusions of an increasing bottom-up control with depth would be reinforced. An overestimation of the production would have the opposite effect. However, it

should somehow be reduced by the decrease of growth yield parallel to the decrease of growth rate with increasing depths as noted above.

Other indices of bottom-up or top-down control

The size of bacteria, the renewal time of bacterial biomass and the proportion of INT reducing cells have been used above as indices of bottom-up control by resource limitation. However, other environmental factors can modify these parameters.

Hagström and Larsson (1984) argue that temperature, another factor of bottom-up control, may affect bacterial cell sizes. Moreover, in some circumstances, a small cell size may be the result of grazing impact (Krambeck, 1988; Gonzalez *et al.*, 1990). This may explain why no evident relationship between cell size and trophic gradient was observed by Van Es and Meyer-Reil (1982), Bird and Kalff (1984), Letarte and Pinel-Alloul (1991), and Tumber *et al.* (1993). Cell size clearly reflects the trophic state of the environment where bacterivores are scarce and temperature variations low.

Several studies have shown that biomass renewal rates are positively correlated with temperature (Hagström and Larsson, 1984; Pomeroy and Deibel, 1986; Shiah and Ducklow, 1994). Therefore, biomass renewal rates may not be considered as evidence of bottom-up control by nutrients without considering the temperature.

Tetrazolium reduction is also positively correlated with temperature (Packard *et al.*, 1975; Rao *et al.*, 1984). This might explain why the percentage of active cells at the oligotrophic site and at the eutrophic site (with a lower temperature) are similar.

The deep sea is ultra-oligotrophic as only a small fraction of photosynthetically produced carbon reaches the layers below 1000 m (Hargrave, 1984; Jannash and Taylor, 1984; Hobbie, 1988). This increasing nutrient control with depth should be reinforced by the combined effects of low temperature, as mentioned above, but also high pressure, generally considered as slowing microbial activities (Jannash and Taylor, 1984; Turley, 1993). Therefore, the influence of temperature and pressure suggests that the size of bacteria, the renewal time of bacterial biomass and the proportion of INT reducing cells should be considered as indices rather than assessments of nutrient control in this study.

Simultaneously with bottom-up control, factors of top-down control, mainly sedimentation, viral lysis and grazing, may have an influence on bacterioplankton biomass. The proportion of large and potentially sinking particles in oligotrophic waters is smaller than it is in eutrophic surface oceanic waters (Cho and Azam, 1988; Simon *et al.*, 1990). A decrease of the abundance of large sinking particles and attached bacteria from surface to deep layers was also reported (Hargrave, 1984; Cho and Azam, 1988). Therefore, sedimentation losses should decrease from the coast to the open ocean and from the surface to the bottom. This is consistent with our observation of increasing bottom-up controls along the same gradients.

On average 10–20% of the total mortality of bacteria may be due to viral infection in marine waters (Suttle, 1994). Viral infection appears to decrease with oligotrophy (Proctor and Fuhrman, 1992; Suttle, 1994). Therefore, viral lysis should decrease, according to the level of the bacterial biomass, from the coastal to the open ocean and from the surface layers to the deep layers.

Such a bacterial density dependence is also well known for grazing. Net growth of bacterivores decreases with bacterial abundance and ceases under a threshold of *ca* 10^8 bacteria l^{-1} (Davis and Sieburth, 1984; Caron *et al.*, 1985; Rivier *et al.*, 1985). Although

the threshold value would likely change among flagellate populations or environments, grazing should not be an important factor of bacterial control below 100 m, where bacterial density falls below 1 to 3×10^8 bacteria l^{-1} (Fig. 1). In surface waters, predatory control should decrease from the eutrophic coastal site ($> 3 \times 10^9$ bacteria l^{-1}) to the oligotrophic site (6×10^8 bacteria l^{-1}).

Therefore, our results are in close agreement with literature data about the control of bacterial biomass by temperature, pressure, sedimentation, viral and grazing losses. Indeed, bacterial biomass is bottom-up controlled; and this control increases with a corresponding decrease in the factors regarding top-down control, from the coastal to the open ocean and from surface to deep layers.

Controls at different space and time scales.

As noted by Pace and Cole (1994), a complication of the model presented by Billen *et al.* (1990) is the situation where predation on bacteria is a major mechanism of nutrient regeneration. When such nutrients are in short supply, most of their regenerated form will be back-assimilated by the bacteria (Caron and Goldman, 1990). In such situations, bacteria may be considered either bottom-up or top-down controlled by grazing. The two controls are tied together. Bacterivore abundance and activity were not determined during this cruise; it is therefore impossible to assess the importance of such retroactive processes at different sites and levels.

Moreover a dominant bottom-up control for bacterial biomass does not exclude a dominant top-down control on time and space scales not considered here. According to Psenner and Sommaruga (1992), shifts between bottom-up and top-down control may occur quickly. Kirchman *et al.* (1993) reported that the average growth rate in the subarctic Pacific is set by a combination of bottom-up controls such as temperature and dissolved organic-matter supply but that daily differences are due to variations in grazing pressure. In some studies, bacterial abundance is clearly modulated by bacterivory over diel cycles (Wikner *et al.*, 1990; Psenner and Sommaruga, 1992), and several other works have shown that heterotrophic nanoflagellates and small ciliates may ingest a large fraction of bacterial production over scales of hours to weeks (Davis and Sieburth, 1984; Caron *et al.*, 1985; Ducklow and Hill, 1985; Rassoulzadegan and Sheldon, 1986; Bjornsen *et al.*, 1988). At mid-latitudes, seasonal shifts between nutrient, temperature and predation controls are reported by Ducklow (1992) and Shiah and Ducklow (1994). However, on large space scales, between systems, regions or basins, or over the whole water column, the variations in nutrient availability or other bottom-up factors (temperature, pressure) are so large that they obscure the top-down forces. Indeed, bacterial populations exceed 10^{10} cells l^{-1} in the most eutrophic coastal lagoons and estuaries, average some 10^9 cells l^{-1} in the coastal zone, range from 0.1 to 1×10^9 cells l^{-1} in the open ocean (Ducklow and Carlson, 1992) and fall to some 10^7 cells l^{-1} in the ultra-oligotrophic deep sea, whatever the importance of grazing, viral loss or sedimentation rate.

CONCLUSIONS

This study has extended the data sets provided by Billen *et al.* (1990) and Ducklow (1992) using the same approach. The use of this approach together with indices of bottom-up or top-down controls such as cell sizes, growth rates, per cent active cells, regression fit between

bacteria and phytoplankton and other indices from the literature suggest a moderate and increasing bottom-up control of bacterioplanktonic biomass from the coast to the open ocean and from the surface to the deep sea, *i.e.* from eutrophic to oligotrophic situations. This conclusion is consistent with the hypothesis of Sanders *et al.* (1992) that bacteria should be more strongly regulated by predation in eutrophic systems.

With respect to such an increasing bottom-up control with oligotrophy, we should have expected the steepest slope of the regression between biomass and production at the oligotrophic site and the weakest at the eutrophic site. Instead of this, similar slopes were obtained regardless of the trophic status (Table 1). This finding should argue for an equal intensity of bottom-up control through the trophic gradient. An alternative explanation could be that the increase of bottom-up control with oligotrophy is partially masked by the stimulation of bacterial production possibly induced by nutrient regenerated from grazing activity (Pace and Cole, 1994). This would tend to lower the slope of the regression as the influence of this nutrient release would likely decrease with increasing top-down control and eutrophy. Actually, an increase in bacterial biomass with bacterial production is consistent with a model where growth is determined by substrate below saturating levels and mortality is of first-order kinetics with respect to biomass (Billen *et al.*, 1990). The situation hypothesized by Pace and Cole (1994), *i.e.* that substrate supply could be directly coupled to mortality, shows that this may not always be observed. Finally, the dominant bottom-up control observed during that study does not exclude a dominant top-down control at other space and time scales not investigated here.

In all cases, we agree with Ducklow (1992) that complex trophic food-web modeling, including many positive and negative feedbacks, would have to demonstrate the correlations between bacterial biomass and production under a situation of bottom-up control.

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