Diel periodicity of bacterioplankton in the euphotic zone of the subtropical Atlantic Ocean

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ABSTRACT: We tested the hypothesis that in the euphotic zone of oligotrophic oceanic waters, the growth-limiting nutrients for bacterioplankton vary over a diel cycle due to competition with phytoplankton, leading to distinct diel patterns in bacterioplankton. During a cruise across the subtropical Atlantic Ocean, a distinct diel periodicity in bacterioplankton was detectable, with highest bacterial abundance in the early morning and declining over the day. A peak in the frequency of dividing cells (FDC, 20 to 25% of the total bacterial community) was recorded at midnight; FDC rates were consistently low during the day and cell volume increased over the day until the early night period. No diel variations in these parameters were detectable in the layers below the deep chlorophyll maximum (DCM). Bio-assay experiments with freshly collected, N+P amended water were started in the early morning. No significant bacterial growth was detectable in the early morning while, in the bio-assays that started at noon, significant bacterial growth was detectable in the N+P treatments. P amendment alone also caused an increase in bacterial abundance although to a lesser extent. Thus, we have evidence that the bacterioplankton in the euphotic layer of the subtropical Atlantic are not limited by the availability of N+P in the morning (most likely limited in C) but at noon when they are competing with phytoplankton for the inorganic N+P sources. Based on the concomitantly measured decline in inorganic N+P in the bio-assays, we estimated the C:N:P ratio of newly produced bacteria. The mean atomic C:N:P ratio for the bacteria in the N+P amended bio-assay experiments started in the early morning was C:N:P = 15:13:1 while the corresponding ratio for bacteria in the bio-assays started at noon was C:N:P = 118:11:1. This diel variation in the C:N:P ratio was caused by the 1 order of magnitude higher bacterial C production at noon as compared to the early morning. Thus, excess uptake of N+P occurs during the morning and luxury C uptake during noon. This plasticity in the C:N:P ratio of bacterioplankton might be a strategy to overcome, at least partly, periods of shortage in a specific nutrient species in the oligotrophic subtropical Atlantic.

KEY WORDS: Atlantic Ocean · Deep chlorophyll maximum · Bacteria · Bacterial growth · Diel periodicity

INTRODUCTION

Bacterioplankton biomass dominates over phytoplankton biomass even in the euphotic zone of oligotrophic open oceans (Fuhrman et al. 1989, Herndl 1991), frequently comprising more than 50% of the particulate organic carbon (POC) present in the system (Cho & Azam 1990). Bacterial abundance in the open ocean is highest in the euphotic zone and declines by about 2 orders of magnitude at depths >1000 m (Ducklow & Carlson 1992, Ducklow et al. 1995, Carlson et al. 1996). This decline in bacterial abundance is accompanied by only a 2- to 3-fold decline in the concentration of dissolved organic carbon (DOC) from the surface waters to the deep ocean. Even if a considerable fraction of the bacterioplankton in the upper mixed layer is inactive (Zweifel & Hagström 1995, Choi et al. 1996,

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Heissenberger et al. 1996, Karner & Fuhrman 1997, Vosjan & Noort 1998), the ratio between bacterioplankton production and DOC concentration is lower in the deep ocean as compared to the surface waters. This implies low turnover rates of DOC in the deep ocean and lower loss rates for the deep water bacterioplankton. In the surface waters, the available DOC supports considerable bacterial activity, resulting in comparatively low turnover times of bulk surface water DOC ranging from <50 d (Kirchman et al. 1991) to <100 d (Carlson & Ducklow 1995, Carlson et al. 1996).

This readily utilizable DOC is mainly derived from phytoplankton extracellular release (Bell & Sakshaug 1980, Bell 1983, Lignell 1990, Williams 1990, Sundh 1992a,b, Obernosterer & Herndl 1995, Biddanda & Benner 1997) and from the grazing activity of zooplankton on phytoplankton (Riemann et al. 1986, Peduzzi & Herndl 1992). Both phytoplankton primary production and zooplankton grazing activity exhibit distinct diel patterns. Thus, if bacterioplankton depend on photosynthetic extracellular release of phytoplankton, one might assume that bacterioplankton also exhibit this distinct diel periodicity in activity. Likewise, if bacterioplankton depend on the DOC released by zooplankton via 'sloppy feeding' (Lampert 1978), then higher bacterial activity might be observed during the night. The diel growth pattern of bacterioplankton in the upper mixed layer of the oligotrophic ocean, however, might be also influenced by physical factors such as ultraviolet radiation (Herndl et al. 1993, Aas et al. 1996).

There are several reports on diel periodicity in bacterioplankton activity following, with some time delay, phytoplankton activity (Sieburth et al. 1977, Burney et al. 1982). Higher bacterial activity has been detected in the late afternoon in a number of coastal seas (Car-

lucci et al. 1984, Fuhrman et al. 1985, Gocke et al. 1987, Herndl & Malacic 1987, Gasol et al. 1998). Johnson et al. (1981), investigating the diel fluctuations of CO_2, oxygen and DOC in the water column of the tropical Atlantic, related the observed diel variations in these parameters to microbial activity. They found accumulation of DOC until late afternoon which was attributed to phytoplankton extracellular release (Johnson et al. 1981). A similar diel pattern in DOC was found in the upper mixed layer of the northern Adriatic Sea (Herndl & Malacic 1987). Again, highest bacterioplankton activity was observed in the late afternoon while in the pycnocline layer, no such periodicity in bacterioplankton activity was detectable. As evident from the above-cited papers, almost all our information on diel periodicity in bacterioplankton activity is derived from studies performed in shelf areas where generally higher overall productivity can be expected as compared to oceanic regions. Under oligotrophic, open ocean conditions, however, the coupling of bacterioplankton to phytoplankton activity might be even more pronounced. Under such conditions, bacterioplankton might then also compete with phytoplankton for the available inorganic nitrogen and, especially, for phosphorus, if the supply of organic phosphorus is not sufficient to support bacterioplankton growth. The competition for phosphorus between phyto- and bacterioplankton is of particular relevance since bacteria with a C:N:P ratio of about 50:10:1 (Fagerbakke et al. 1996) need relatively more phosphorus than phytoplankton with a C:N:P ratio of 106:16:1 (Redfield et al. 1963).

This competition between phyto- and bacterioplankton for inorganic nutrients and the trophic dependence of bacterioplankton on phytoplankton exudates might therefore be most pronounced in the oligotrophic, (sub)tropical ocean, and particularly in the upper mixed layer above the deep chlorophyll maximum (DCM) layer. This hypothesis was tested during a cruise in the subtropical Atlantic in the summer of 1996. As a consequence of this tight coupling between phyto- and bacterioplankton, we furthermore hypothesized that the elements limiting bacterioplankton growth also vary over a diel cycle, thus reflecting the competition between phyto- and bacterioplankton. Since we expected this interaction between phyto- and bacterioplankton to be most pronounced in the mixed layer above the DCM we compared the diel pattern in bacterioplankton activity of these 2 layers.
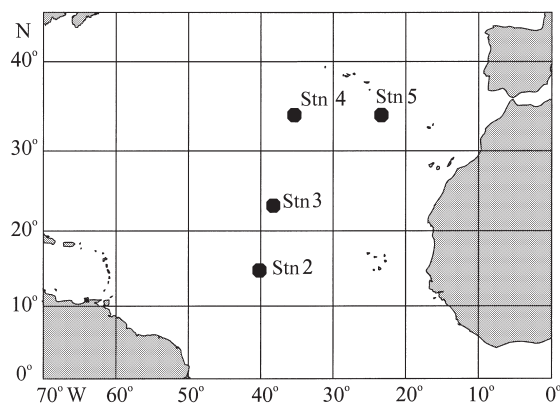

Fig. 1. Sampling stations in the Atlantic Ocean during August 1996. Stations were occupied between 5 and 9 August (Stn 2), 11 and 15 August (Stn 3), 18 and 22 August (Stn 4), 24 and 28 August (Stn 5)

MATERIAL AND METHODS

Description of the sampling sites. The study was carried out during a 6 wk research cruise devoted to

the DCM layer of the subtropical Atlantic Ocean on the RV 'Hr. Ms. Tydeman' (Fig. 1). Five stations, each occupied for 4 d, were sampled along a transect during August 1996. Since Stn 1 (12° N, 48° W) was still under the influence of the South American continental shelf, we report here only results from the other 4, truly oceanic stations (Stn 2: 14° N, 40° W; Stn 3: 23° N, 38° W; Stn 4: 34° N, 35° W; and Stn 5: 34° N, 23° W).

At each of the fixed stations, series of CTD hydrocasts (SeaBird) were performed over diel cycles and water samples were taken with 10 l NOEX bottles mounted on a rosette from 30 and 200 m depth and from 3 depths around the DCM layer. Because the depth of the DCM varied due to internal waves, samples for the DCM layer were taken at the depth of the maximum fluorescence and at the upper and lower boundaries at approximately half of the maximum fluorescence. Water samples were collected early in the morning, at noon, in the evening and at midnight. As soon as the CTD rosette was on board the ship, water was carefully collected for the determination of inorganic nutrients, bacterial and flagellate enumeration, POC and for the bio-assay experiments as described below.

Bio-assay experiments to determine the growth-limiting element for bacterioplankton. For logistic reasons, only 1 depth layer was sampled for the bioassay experiments per day, either in the morning or at noon. Per sampling, 6 l of seawater was filtered through 0.6 µm filters (Nuclepore, polycarbonate). This 0.6 µm filtered water was examined for the presence of autofluorescent cells by flow cytometry (Coulter). Flow cytometric analysis indicated that filtration through 0.6 µm efficiently removed all autofluorescent particles (phytoplankton) while almost all the bacteria passed through the filter. Thereafter, the 0.6 µm filtered seawater was dispensed into 6 rinsed 1 l (1 N HCl) polycarbonate bottles and either left unamended or amended with either carbon, nitrate or phosphate or with a combination of nutrients. As a carbon source, a stock solution consisting of glucose (2.5 g l^{-1}), pyruvate (3.1 g l^{-1}) and acetate (3.4 g l^{-1}) was prepared and 1 ml of this carbon stock solution was added to the respective 1 l flasks, resulting in a final amendment of 1 mg C l^{-1}. Nitrate was added in the form of NaNO$_3$ (0.2 ml of 50 mM NaNO$_3$), resulting in a 10 µM N enrichment in the 1 l flasks. Phosphate was added as K$_2$HPO$_4$ (0.5 ml, 2 mM) to result in a final concentration of 1 µM P in the flasks. Filtration through 0.6 µm led to an increase in the NH$_4$ and PO$_4$ concentration in the filtered seawater by, on average, 108 ± 37 and 162 ± 66 % (n = 19), respectively, as compared to the raw seawater prior to filtration (see Table 1). This filtration-induced enrichment in N and P, however, was negligible for our bio-

assay experiments since we increased the nutrient levels in the respective nutrient additions by at least 1 order of magnitude.

All flasks were mounted on a slowly rotating plankton wheel (0.5 rpm) incubated in a temperature-controlled incubator in the dark at *in situ* temperature (~26°C) until maximum bacterial abundance was reached (generally within 24 h). After amending the different flasks with the respective nutrients, 5 ml subsamples were taken from the different treatments for bacterial enumeration and 20 ml for the determination of the concentrations of inorganic nutrients at 4 to 6 h intervals (described below).

Bacterial and flagellate enumeration. For enumeration and cell volume determinations of the bacterioplankton from the different depths of the water column and the different bio-assay treatments, 5 ml subsamples were fixed with 2 % formaldehyde (final concentration), stained with acridine orange for 4 min and then filtered onto black Nuclepore filters (0.2 µm pore size). The filters were mounted on slides embedded in paraffin oil and stored frozen at –20°C. Enumeration of the bacteria was performed with an epifluorescence microscope (Zeiss Axioplan) at 1250× magnification (Hobbie et al. 1977). At least 10 fields or 200 bacteria per sample were counted. Additionally, the cell volume of the bacteria was determined on each sample using an image analysis system (Leica G570) linked to the epifluorescence microscope. At least 100 bacteria per sample were sized and the volume calculated. Carbon biomass of the bacterioplankton community was calculated using the relation between cell volume and carbon content per cell given in Lee & Fuhrman (1987). Moreover, the frequency of dividing cells (FDC) was determined for the bacterioplankton community in different depth strata of the water column while enumerating bacterial abundance (Hagström et al. 1979). Only those bacteria were counted as dividing cells showing a clear invagination.

In order to determine the abundance of heterotrophic nanoflagellates (HNAN) in the water column, 10 ml subsamples were fixed with formalin (2 % final concentration) and stained with proflavine (Caron 1983). Seventy-five fields were enumerated per sample under the epifluorescence microscope.

POC determination. Ten litres of water collected from the respective depth was filtered onto a combusted (450°C for 4 h) pre-weighted Whatman GF/F filter, dried on board the ship at 70°C for 6 h and stored in a desiccator. In the laboratory, the dry weight of the sample was determined with a Mettler AT 261 precision balance. Subsequently, the organic C was determined using a C-H-N elemental analyzer (Carlo Erba 1500) after acidifying the sample with 0.5 ml of 2 % HCl to remove inorganic C.

Inorganic nutrient analysis. Inorganic nutrient analyses of water collected from distinct sampling depths and the bio-assay experiments were performed on board the ship using standard methods (Parsons et al. 1984) and a TRAACS autoanalyzer system.

Statistical analysis. Unless stated otherwise, the variables from the different depth strata (mixed upper layer, DCM, layer below the DCM) were compared by pooling the data from all 4 stations according to the depth strata. ANOVA and Tukey HSD multiple comparison were then performed to test for differences between the depth strata.

RESULTS

Diel pattern of fluorescence and bacterial abundance

The depth of the DCM layer varied between 70 and 140 m but was typically at about 100 m at all stations over the diel cycles (Fig. 2). The intensity and the distribution pattern of the phytoplankton fluorescence were rather stable throughout the diel cycles at each station; however, differences between stations were detectable. Bacterial abundance exhibited consistent diel fluctuations (Fig. 2) and was generally significantly higher in the morning with a peak at around 50 m depth at all stations ranging from 1.1×10^6 ml^{-1} (Stn 4) to 1.4×10^6 ml^{-1} (Stn 3) as compared to the bacterial abundance during the rest of the day (noon, afternoon and midnight; ANOVA, Tukey HSD multiple comparison, p < 0.001). This peak in bacterial abundance was always detectable above the DCM layer. From noon until evening, bacterial abundance

was more evenly distributed in the upper 100 m of the water column and was constant below the euphotic zone, ranging from 0.2×10^6 ml^{-1} to 0.42×10^6 ml^{-1} in the 150 to 200 m depth layer at all stations with no diel pattern detectable (ANOVA, p = 0.798). Mean bacterial cell volume was generally lower in the morning and noon than at midnight (ANOVA, p < 0.02) in the upper mixed layer and the DCM (ANOVA, p < 0.03) (Fig. 2). As for bacterial abundance, no significant diel variations in bacterial cell volume were detectable in the layers below the DCM (ANOVA, p = 0.818).

Diel pattern of FDC

Pronounced diel patterns in FDC were found in the mixed and the DCM layers with no significant difference in the pattern of the FDC between both layers (ANOVA, p = 0.591) (Fig. 3). At all the stations, highest FDC was found at midnight and dropped significantly towards the morning (ANOVA, Tukey HSD multiple comparison, p < 0.0001) in both layers (Fig. 3). No significant differences in the FDC were found between the morning, noon and evening (ANOVA, Tukey HSD multiple comparison, p = 0.466) in both layers.

Diel fluctuation in HNAN abundance

Similar to the distribution of bacteria, HNAN exhibited a significantly higher abundance in the upper mixed water column and the DCM in the early morn-

Table 1. Concentrations of inorganic N and PO$_4$ (in µM) at the different stations in the Mixed and the deep chlorophyll maximum (DCM) layers. This water was also used for the bio-assay experiments after amending it with the respective nutrients. Detection limits are 0.1 µM for NO$_3$ and NO$_2$, 0.08 µM for NH$_4$, and 0.015 µM for PO$_4$

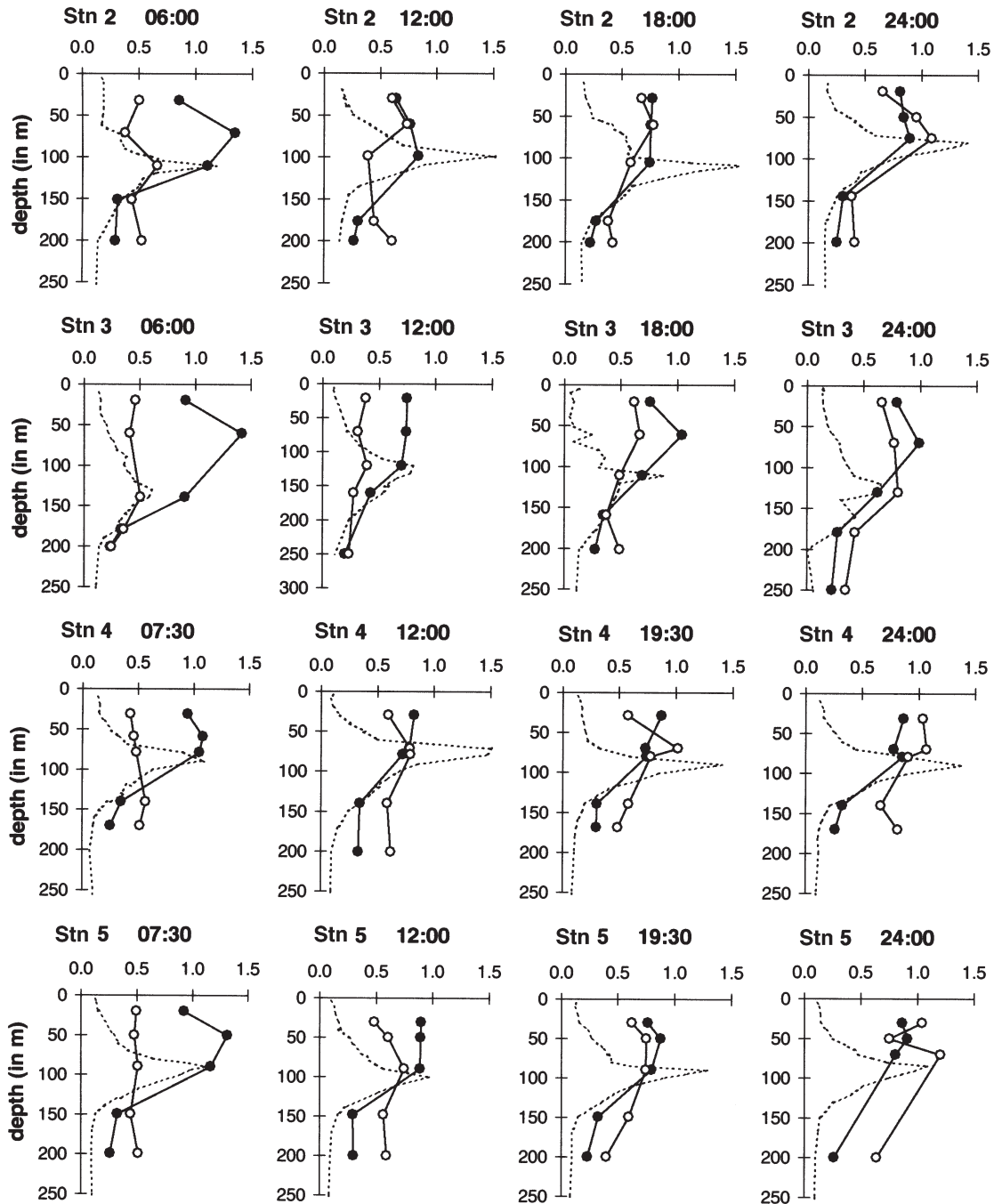| Time (h)	Stn	Layer	NO$_3$	NO$_2$	NH$_4$	PO$_4$
07:03	2	Mixed	<0.1	<0.1	0.147	0.033
07:04	2	DCM	0.577	0.100	0.150	0.078
07:00	3	Mixed	<0.1	<0.1	0.136	<0.015
05:54	3	DCM	<0.1	<0.1	0.202	0.060
07:21	4	Mixed	<0.1	<0.1	0.170	<0.015
07:03	4	DCM	<0.1	<0.1	0.140	0.016
07:02	5	Mixed	<0.1	<0.1	0.319	0.017
06:03	5	DCM	0.379	<0.1	0.181	0.020
12:34	2	Mixed	<0.1	<0.1	0.229	0.018
12:06	2	DCM	0.137	<0.1	0.154	0.088
12:34	3	Mixed	<0.1	<0.1	0.127	<0.015
12:34	3	DCM	<0.1	0.10	0.156	<0.015
12:09	4	Mixed	<0.1	<0.1	0.118	<0.015
12:28	4	DCM	0.398	0.133	0.210	0.020
12:29	5	Mixed	<0.1	<0.1	0.272	0.020
12:01	5	DCM	<0.1	<0.1	0.208	0.037

Fig. 2. Distribution of phytoplankton fluorescence (---), bacterial abundance (●, $n \times 10^6$ ml^{-1}) and cell volume (○, $\times 0.2$ μm^{-3}) in the upper 200 m of the water column at the 4 stations over a diel cycle

ing (ANOVA, $p < 0.03$) than during the rest of the day (Fig. 4). The lowest HNAN abundance was detected around noon, and thereafter increased again. Generally, HNAN abundance ranged from ~1.5 to 3.8 $\times$ 10^3 ml^{-1} in the euphotic zone and declined to 0.3×10^3 ml^{-1} at 200 m depth with no diel variation detectable (ANOVA, $p = 0.469$) (Fig. 4).

Fluctuations in the concentrations of inorganic nitrogen, phosphorus and POC

At all sampling sites, large diurnal variations in the inorganic nitrogen and phosphorus concentrations were detectable (Table 1). In the mixed layer at Stn 2, inorganic nitrogen increased from the morning
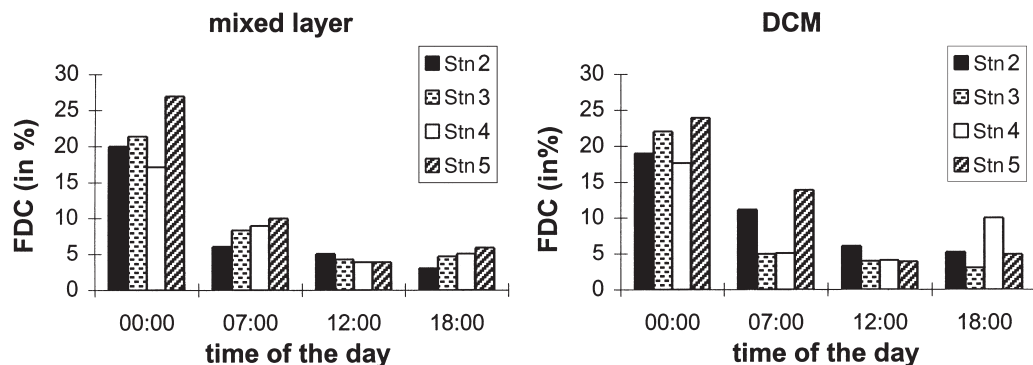
mixed layer

DCM

Fig. 3. Distribution of the frequency of dividing cells (FDC) of total bacteria in the upper mixed layer and in the deep chlorophyll maximum (DCM) over a diel cycle

to noon by about 80% while at the same time, inorganic phosphorus declined by 45% (Table 1). Even larger variations between morning and noon were detected for the DCM layer at Stn 3. The atomic ratio between inorganic N:P varied between 4.6 (Stn 2,

morning) and 44.3 (Stn 3, noon). Thus, considerable variations in the ratios of growth-limiting macro-nutrients were observed with no consistent diurnal pattern discernible for either the mixed or the DCM layer.
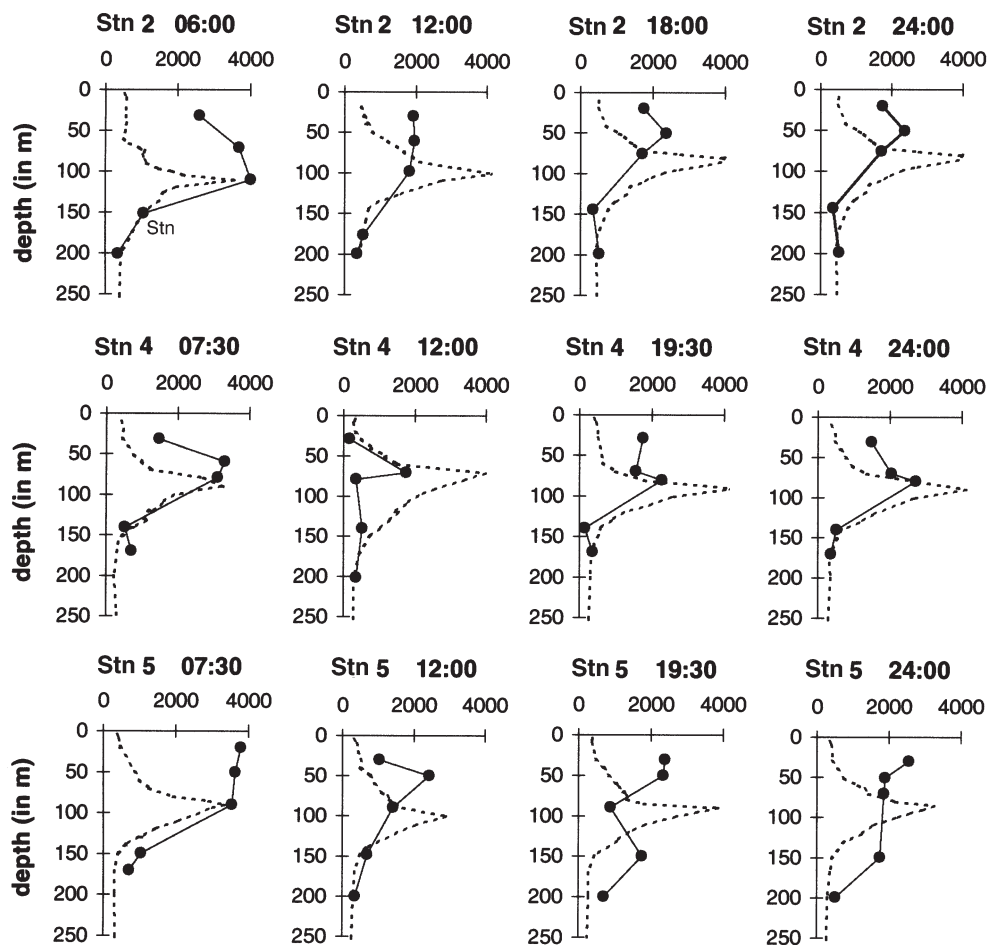

Fig. 4. Diel dynamics in the abundance of heterotrophic flagellates (●, ml^{-1}) in the upper 200 m water column at 3 stations. For comparison the relative fluorescence (- - -) is also given. For Stn 3, no data are available due to logistic problems

POC concentrations varied only over a small range during the diel cycles at the individual stations and generally increased slightly from the surface layers to the upper boundary of the DCM layer (Fig. 5). The bacterioplankton contribution to the POC pool varied between 40 and 80% in the mixed layer and the DCM and declined rapidly to 10% below the DCM (Fig. 5).

Bio-assay experiments

Bio-assay experiments revealed different growth responses of the bacterioplankton community depending on the nutrient species added and the time of the day (morning vs noon) when the water was collected (Fig. 6). In the morning, maximum bacterial abundance in the treatments amended with N+P was not significantly higher than that in the unamended controls, for water collected both from the mixed layer and from the DCM (Student's t-test, $p = 0.144$, for both layers). N+P amendment, however, led to a significantly higher bacterial abundance in the waters collected at noon as compared to the N+P amendment in the morning (Student's t-test, $p < 0.0001$). Neither C nor N amendment significantly increased bacterial abundance in the incubations started at noon while, on average, P addition alone stimulated bacterial growth (except for Stn 4, mixed layer, and Stn 5, DCM) to a level similar to the N+P amendments. Generally, the highest bacterial abundance was reached when C+N+P was added.

DISCUSSION

Throughout the transect across the subtropical Atlantic, distinct diel patterns in bacterioplankton abundance were observed with significantly higher bacterial abundance in the early morning as compared to the other times of the day (Fig. 2). This pattern in bacterial abundance was detectable only in the euphotic zone and corresponds to the distribution of the FDC in the mixed layer and the DCM (Fig. 3). FDC was highest at midnight and lowest at noon. The average cell volume of the bacteria reflects this pattern in cell division as well. The lowest cell volume was detected in the morning and increased consistently during the day, with the highest cell volume reached at midnight, when the FDC was also highest. Thus, despite the expected heterogeneity of the species composition of the bacterioplankton in the subtropical Atlantic (Giovannoni et al. 1990, Mullins et al. 1995), there is a clear diel pattern in bacterial cell division detectable, indicating phasing of growth. Such diel
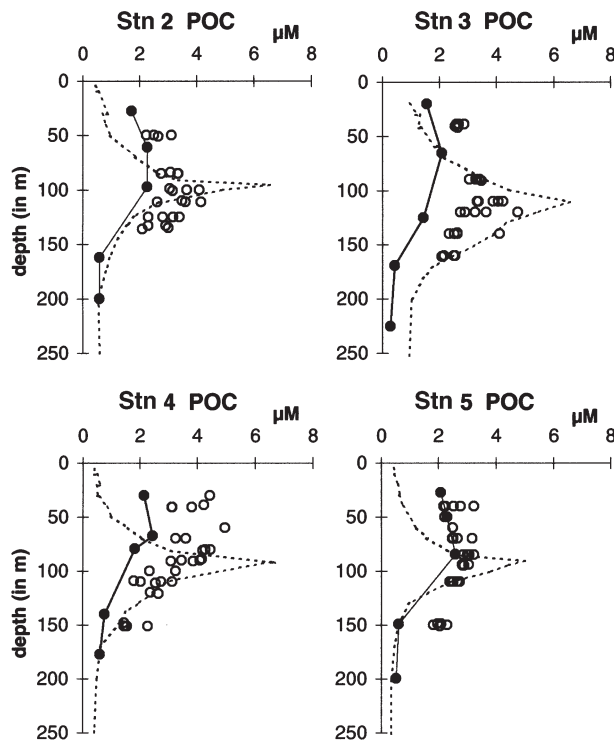

Fig. 5. Concentration (in µM C) of total particulate organic carbon (POC, ○) and the average bacterial C biomass (●) derived from enumeration under the epifluorescence microscope. For comparison the fluorescence is also indicated (---)

growth patterns have been reported previously for a number of coastal and open ocean regions and lentic systems (Riemann et al. 1984, Riemann & Søndergaard 1984, Fuhrman et al. 1985, Herndl & Malacic 1987, Zweifel et al. 1993, Simon 1994, Gasol et al. 1998). Most of these studies used the incorporation of radiolabeled thymidine and/or leucine to determine the diel growth pattern.

From the information available from a diverse range of aquatic habitats, it appears that whenever diel periodicity in bacterial activity is detectable in surface waters, bacterioplankton show peaks in leucine uptake from noon to late afternoon (Zweifel et al. 1993, Gasol et al. 1998). Thymidine incorporation tends to be higher in the early morning than in the afternoon (Riemann & Søndergaard 1984, Zohary & Robarts 1992, Simon 1994, Gasol et al. 1998), although this pattern is less pronounced than that for leucine. Generally, largest diel variations in bacterial activity are found under oligotrophic conditions (Zohary & Robarts 1992, Zweifel et al. 1993, Gasol et al. 1998, this study), while under more eutrophic conditions the diel patterns in bacterial activity are not that pronounced (Riemann & Søndergaard 1984, Zweifel et al. 1993, Simon 1994). Differences in the diel periodicity of bacterial activity
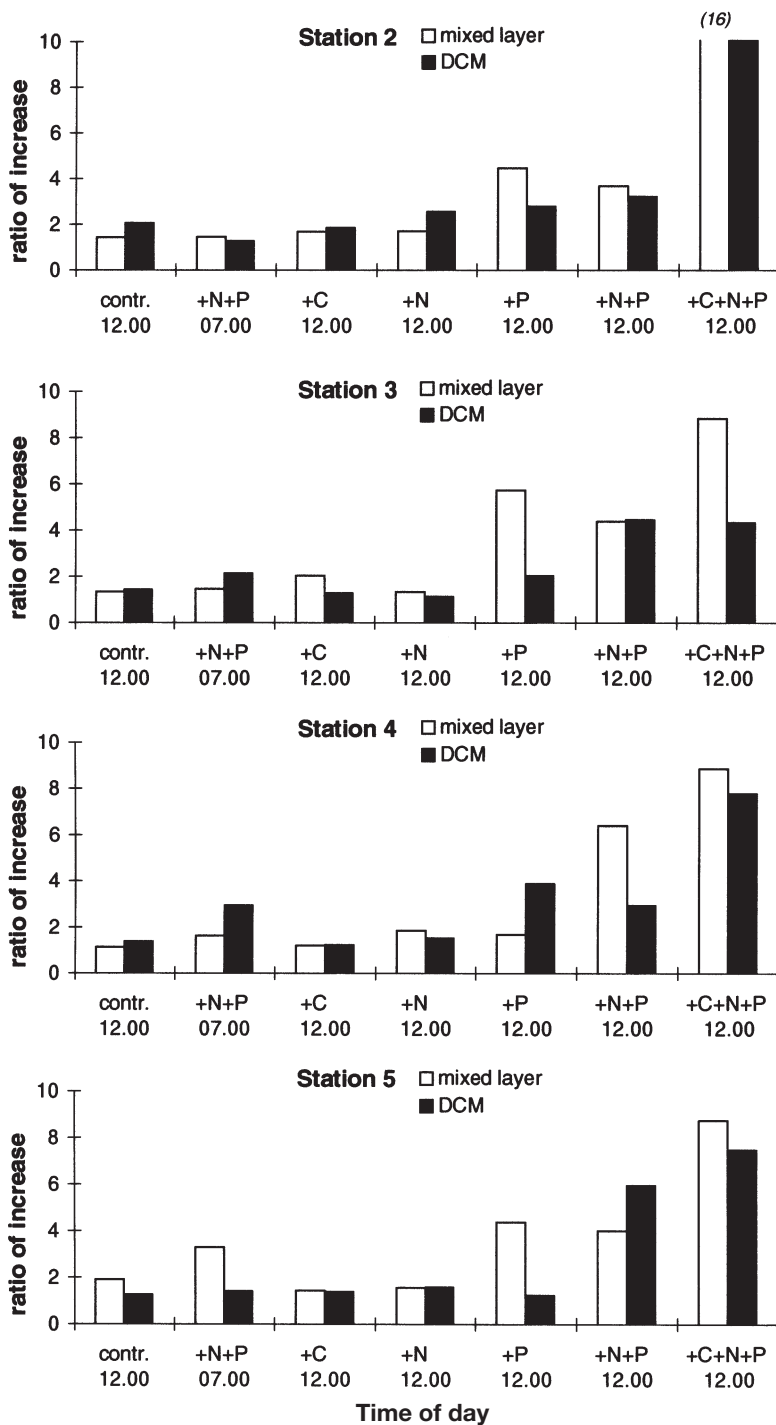
Fig. 6. Growth response of bacterioplankton expressed as the ratio of maximum:initial abundance in the different treatments of the bio-assays using water from the upper mixed layer and the DCM. Bio-assay experiments started at 07:00 and 12:00 h local time

have been reported between the upper mixed layer, where pronounced diel activity patterns are frequently detectable, and boundary layers such as pycnoclines or DCM layers, where the diel fluctuations in bacterial

activity are usually lower (Herndl & Malacic 1987, Gasol et al. 1998). In the present study, the diel fluctuations in bacterial abundance in the upper mixed layer were of similar amplitude than in the DCM (Fig. 2) and no differences in the diel dynamics of the FDC were found between the 2 layers (Fig. 3).

What causes this persistent diel growth pattern or, in another term, the strong phasing in growth of bacterioplankton in the oligotrophic subtropical Atlantic? Phasing of growth (also referred to as unbalanced growth) has been frequently observed, mostly by measuring the bacterial incorporation of leucine versus thymidine (Chin-Leo & Kirchman 1990). Besides some unspecific incorporation of thymidine into bacterial macromolecules other than DNA, larger changes in the ratio between leucine and thymidine occur when bacterial communities shift from high to low growth rates and vice versa (Chin-Leo & Kirchman 1990). Using a different approach (abundance fluctuations, cell volume of the cells, FDC), we observed phasing of bacterial growth, thereby avoiding the problems inherent to the commonly used radiolabeling techniques (leucine and thymidine incorporation) of unspecific incorporation. From the pattern of bacterial abundance, average cell volume and FDC we can deduce that bacterioplankton divide during the night followed by an increase in abundance in the morning and an increase in cell volume during the day (Figs. 3 & 4). Thus, a clear relation exists between the increase in cell volume and phytoplankton activity in the mixed layer and, less pronounced, in the DCM.

During daytime, phytoplankton primary production might lead to an increase in DOC available for bacterioplankton growth. Diel fluctuations in labile DOC in the upper oceanic water column with higher concentrations in the afternoon have been shown repeatedly, attributed to phytoplankton activity and the release of photosynthetically fixed carbon, mainly amino acids and carbohydrates (Meyer-Reil et al. 1979, Johnson et al. 1981, Burney et al. 1982, Fuhrman & Ferguson 1986, Leboulanger et al. 1997). Bulk DOC measurements, however, are certainly too

crude to reflect subtle diel changes in the concentration of labile DOC, which are in the nanomolar range for amino acids and carbohydrates. This frequently observed accumulation of specific components of the DOC pool in the afternoon also indicates that bacteria in the upper water column are not capable to take up this labile DOC pool completely when its release increases. This might be due to partial inhibition caused by ultraviolet (UV) radiation in these sunlit oligotrophic waters, where the 10% radiation level of 340 and 380 nm wavelength is at 35 and 60 m depth, respectively (Obernosterer et al. in press). Thus, about half of the euphotic layer is exposed to potentially harmful UV radiation, which affects bacterioplankton more than phytoplankton (Jeffrey et al. 1996) and leads to an accumulation of DNA damage in bacterioplankton cells during the period of intensive solar radiation. Bacterioplankton rapidly recover from this UV-induced DNA damage using photoenzymatic repair mechanisms (Kaiser & Herndl 1997). Thus, the combination of enhanced substrate availability during the daytime and intensive UV radiation might be responsible for the observed increase in cell volume of bacterioplankton during the day in the upper water column. Photoenzymatic DNA repair induced by the wavelength spectrum 370 to 450 nm (Sancar 1996) might allow bacterioplankton to recover from this UV stress in the late afternoon, when solar radiation decreases in intensity and the wavelength spectrum in the water column is shifted towards longer, less-harmful wavelength of radiation. Bacterial cell division is then accomplished mainly in the dark period as shown in Fig. 3, thereby avoiding exposure of DNA to potentially harmful UV radiation during cell division. Using a different approach, Aas et al. (1996) found a similar diel bacterioplankton activity pattern with lower thymidine incorporation, hence DNA synthesis, as compared to leucine incorporation (biomass synthesis) in the presence of UV radiation in the Gulf of Mexico.

In order to increase the average cell volume during the daytime as detected in our study, bacterioplankton might have to compete with phytoplankton for nitrogen, and more importantly, for phosphorus (Dowing 1997). Since the elemental composition of bacteria is roughly C:N:P = 50:10:1 (Fagerbakke et al. 1996) while that for phytoplankton is C:N:P = 106:16:1 (Redfield et al. 1963), bacteria need relatively more P than phytoplankton. It becomes increasingly clear that over vast areas of the open ocean, phytoplankton primary production, as well as bacterioplankton activity, is phosphorus limited (Thingstad & Rassoulzadegan 1995, Cotner et al. 1997, Dowing 1997, Rivkin & Anderson 1997). This phosphorus limitation of bacterial activity might lead to an accumulation of DOC (Obernosterer &

Herndl 1995, Zweifel et al. 1995, Thingstad et al. 1997). All the above-cited papers came to the conclusion that bacterial production might be limited by the availability of inorganic nutrients from enrichment experiments with water collected during daytime. It is reasonable to assume, however, that the limiting nutrient species vary over a diel cycle due to the diel activity pattern of phytoplankton. During daytime, when phytoplankton photosynthesis takes place, bacterioplankton have to compete for inorganic nutrients with phytoplankton, while during the night the competition for inorganic nutrients might be lower. In contrast, during daytime, when phytoplankton production is high, the release of readily available DOC by phytoplankton might fuel the carbon demands for bacteria. Based on these considerations, we determined the diel variability in the limiting nutrient species using bio-assay experiments and water collected during different times of the day.

Almost all the bio-assay experiments performed thus far have used radiolabeled thymidine and/or leucine to determine the bacterial response (Kirchman 1990, Zweifel et al. 1993, Cotner et al. 1997, Rivkin & Anderson 1997). Although convenient, this approach has to be used with caution as discussed recently (Rivkin & Anderson 1997). Briefly, bacterial abundance might increase in these bio-assays without any noticeable increase in thymidine or leucine uptake (Heinänen 1992, Carlson & Ducklow 1996); however, the opposite trend of increasing thymidine and leucine incorporation at stable bacterial abundance has also been reported (Kirchman 1990). Thus, in the present study we used the increase in cell abundance as determined by epifluorescence microscopy to determine the response of bacteria to nutrient additions.

We found distinct patterns in the response of bacteria to nutrient amendments in the bio-assay experiments depending on the time of the day when the bio-assays were started (Fig. 6). If the bio-assays were started in the morning, the bacterial abundance was not significantly higher in the treatments amended with N+P than in the unamended controls. When the bio-assay experiments were started at noon, N+P amendments resulted in significant bacterial growth while C enrichment did not lead to significant bacterial growth (Fig. 6). P amendment alone resulted in a higher bacterial abundance than N amendment in the bio-assay experiments started at noon. The highest bacterial abundance was always detected in the bio-assay experiments started at noon in the treatments amended with C+N+P. Overall, we consistently detected shifts in the growth-limiting elements over a diel cycle, from an obvious C limitation in the early morning to a predominant P limitation at noon.

Using a similar approach, Rivkin & Anderson (1997) recently found that bacterioplankton were C limited in the Caribbean Sea, while in the Gulf Stream and the Sargasso Sea bacteria were primarily P limited in their growth. Cotner et al. (1997) also concluded that bacterioplankton is P limited in the Sargasso Sea. We are not aware of any other study except the present one performing bio-assay experiments on a diel basis and covering a comparable large area of the open ocean. The clear shift from C-limited bacterial growth in the morning to primarily P limitation at noon implies that it might be a successful strategy for bacterioplankton to take up excess substrate when it is available in sufficient quantities and store it for periods when this particular substrate is not available.

Since we measured the time course of the decline of inorganic N and P in the N+P amended treatments of the bio-assays over the incubation period, we can compare the production of bacterial C with the concomitant consumption of inorganic N and P (Table 2). This gives us an estimate of the C:N:P ratio of new-produced bacteria; however, several assumptions have to be made: (1) no other (organic) N and P sources are taken up by bacterioplankton during the incubation, (2) the C content of the bacteria growing in the bio-assay follows the relation between cell volume and C content given in

(Lee & Fuhrman 1987), (3) no consumers of the inorganic nutrients other than bacterioplankton are present in the bio-assays, and (4) the incubations are free of bacterivores. Certainly, other (organic) N+P sources were present in the bio-assays although their uptake rates were probably low compared to the uptake of the added inorganic N+P. The actual C content per cell volume was not measured during this study. There are reports, however, that the C content for open ocean bacteria is relatively constant (Fukuda et al. 1998). The absence of other organisms (particularly of *Synechococcus* and *Prochlorococcus*) has been checked by flow cytometry, no other microorganisms were present in significant numbers. As shown in Table 2, the C production by bacteria was 1 order of magnitude lower in the N+P enriched bio-assays started in the morning than at noon. No significant differences in the N+P uptake were found in the bio-assay experiments started in the morning as compared to those at noon.

The C:N:P ratios of newly produced cells calculated from the increase in bacterial C biomass and the concurrent decline in the inorganic N and P in the bio-assay experiments (Table 2) deviate considerably from the C:N:P ratio of around 50:10:1 reported for bacteria (Fagerbakke et al. 1996). In the N+P enriched bio-assay experiments started in the morning, bacteria

Table 2. Bacterial C production and the inorganic N and P taken up by bacteria in the bio-assay experiments enriched with N+P at the different stations and the 2 depth layers (upper mixed layer, DCM). Bio-assay experiments were started at around 07:00 (morning) and 12:00 h (noon) local time. The bacterial C production was calculated from the increase in abundance from the beginning of the incubation until reaching the stationary phase and the N and P consumption was calculated from the concomitant decrease in inorganic N and P concentrations in the incubation media during the same period. The atomic C:N:P ratio of the newly produced bacteria is also given

Stn	Layer	C produced (μmol C l^{-1} h^{-1})	N consumed (μmol N l^{-1} h^{-1})	P consumed (μmol P l^{-1} h^{-1})	C:N:P
Morning					
2	Mixed	0.0198	0.0171	0.0013	15.7:13.5:1
2	DCM	0.0297	0.0128	0.0013	22.3:9.6:1
3	Mixed	0.0194	0.0192	0.0008	23.8:23.6:1
3	DCM	0.0412	0.0481	0.0019	21.4:25:1
4	Mixed	0.0700	0.0121	0.0118	5.9:1:1
4	DCM	0.1372	0.0524	0.0080	17.2:6.6:1
5	Mixed	0.0590	0.0354	0.0073	8:4.8:1
5	DCM	0.0256	0.0443	0.0028	9.1:15.8:1
Mean		**0.0502**	**0.0302**	**0.0044**	**15.4:12.5:1**
SD		**0.0371**	**0.0157**	**0.0038**	**6.5 8.1**
Noon					
2	Mixed	0.2360	0.0324	0.0026	89.8:12.3:1
2	DCM	0.3108	0.0492	0.0052	59.4:9.4:1
3	Mixed	0.2623	0.0509	0.0032	83:16.1:1
3	DCM	0.2955	0.0246	0.0072	41.1:3.4:1
4	Mixed	0.6151	0.0557	0.0106	57.9:5.2:1
4	DCM	0.2359	0.0147	0.0008	309.8:19.3:1
5	Mixed	0.1673	0.0188	0.0011	151.9:17.1:1
5	DCM	0.8019	0.0389	0.0052	153.7:7.5:1
Mean		**0.3656**	**0.0357**	**0.0045**	**118.3:11.3:1**
SD		**0.2074**	**0.0145**	**0.0031**	**82.3 5.5**

took up N and P in excess as compared to the C produced (C:N:P = 15:13:1) while in the bio-assays started at noon, bacteria produced about 2 times more C biomass (mean C:N:P = 118:11:1) than their concomitant uptake of N and P would suggest (Table 2). Although there is considerable uncertainty in the calculation of the C:N:P ratios, they indicate that bacterioplankton can take up substrate well in excess of their overall C:N:P ratio. This plasticity in the C:N:P ratio of newly produced bacteria might further indicate that bacteria can, at least to some extent, counteract short-term nutrient limitation. However, comparing the growth responses in the bio-assays started in the morning and at noon, it is evident that luxury uptake of N and P in the morning (Table 2) is not sufficient to allow growth exclusively on C substrate provided by phytoplankton extracellular release during the day (Fig. 6). Only after amendment with N+P was the available C taken up in excess of their overall C:N:P ratio. This shortage of available N and P during the day might also be responsible for the frequently observed increase in DOC during the afternoon (Johnson et al. 1981, Burney 1986).

In summary, we have shown that across the subtropical Atlantic, bacterioplankton exhibit a pronounced diel activity pattern in the upper mixed and DCM layers. Bacterioplankton cell numbers were highest in the morning and decreased steadily throughout the day while their cell volume increased concurrently. The bacterial cells divided mostly around midnight and were obviously not limited by the availability of N+P during the night but these elements were growth-limiting during the day. This indicates that growth-limiting elements might change over a diel cycle in the open ocean and that bio-assay experiments designed to determine the growth-limiting nutrients have to take this diel periodicity into account. Furthermore, our results point to a strong diel phasing (unbalanced growth) of bacterioplankton growth probably governed by UV radiation and phytoplankton activity in the oligotrophic subtropical Atlantic.

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