

Diel variations in bacterial heterotrophic activity and growth in the northwestern Mediterranean Sea

Josep M. Gasol^{1,*}, M. D. Doval², Jarone Pinhassi³, Juan I. Calderón-Paz¹,
Núria Guixa-Boixareu¹, Dolors Vaqué¹, Carlos Pedrós-Alió¹

¹Institut de Ciències del Mar, CSIC, Passeig Joan de Borbó, s/n, E-08039 Barcelona, Spain

²Instituto de Investigaciones Marinas, CSIC, Eduardo Cabello 6, E-36208 Vigo, Spain

³Department of Microbiology, Umeå University, S-901 87 Umeå, Sweden

ABSTRACT: Primary producers must respond to the diel changes in light availability. Therefore, detection of diel cycles in bacterial activity would imply tight coupling between the production of photosynthetic dissolved organic carbon (DOC) and its consumption by bacteria. Absence of diel cycles, on the contrary, would indicate that bacteria depend largely upon allochthonous organic carbon and that bacteria are not tightly dependent on photosynthetically produced autochthonous carbon. In 1993 and 1994 we sampled 3 sites in the NW Mediterranean Sea several times a day, and measured several microbial parameters as well as the vertical profiles of DOC along the diel cycle. The sites were selected so that one was on the continental shelf and, thus, was more influenced by coastal runoff; a second one was over the shelf slope and a third, oceanic one was located further offshore over a depth of 2000 m. We found clear diel cycles in bacterial total and specific activity always in the oceanic stations and sometimes in the shelf slope stations. Diel changes were detected as changes in both DNA and protein synthesis rates. These diel cycles were accompanied by diel changes in the distribution of total DOC, and by diel changes in the proportion of bacteria containing visible nucleoids. Noon estimates of bacterial activity were more than twice the daily average in the oceanic site, but they were less different in the other 2 sites. DOC changed daily by 15 μM (5 to 15% of the total stock). For bacterial activity to explain the diel changes in DOC concentration, bacteria should have growth efficiencies lower than 10% in general, and lower than 2% in the oceanic station.

KEY WORDS: Bacterial production · Mediterranean · Diel cycles · DOC diel changes · Nucleoid-containing bacteria · Bacterial carbon conversion efficiencies

INTRODUCTION

Bacteria are important components of the pelagic food web both in terms of biomass and activity. Their role in organic matter processing and nutrient recycling is now well established (Pomeroy 1974, Azam et al. 1983). Bacteria perform the key role of converting dissolved organic carbon (DOC) to particulate carbon (Azam & Hodson 1977). This particulate carbon retains nutrients and becomes available to other trophic levels. Bacteria are also the principal mediators of particle decomposition in the pelagial (Cho & Azam 1988).

Autochthonous production, either directly excreted from the primary producers or through zooplankton excretion and sloppy feeding, is assumed to be the main source of the organic matter used by bacteria in oceanic environments. In coastal areas, however, allochthonous carbon is also processed by bacteria and incorporated into the particulate organic carbon (POC) pool. There is a relatively good relationship between primary and bacterial production across systems (Cole et al. 1988), suggesting that roughly half the carbon produced by algae is processed by bacteria. This suggests that bacteria are mostly dependent on the photosynthetic production of organic carbon, even though little direct evidence is available. To understand the role of bacteria as carbon and nutrient recyclers in planktonic environments, we should determine the

*E-mail: pepgasol@icm.csic.es

way in which bacterial activity is coupled to primary production, and to what extent allochthonous carbon sources and the grazing activity of the zooplankton (Peduzzi & Herndl 1992) participate in providing substrates for bacterial growth. Recent evidence suggests that coupling between primary and bacterial producers in the pelagial of oligotrophic seas is not as tight as it was thought (del Giorgio et al. 1997). The results compiled by del Giorgio et al. (1997) show imbalances in the carbon budget of many unproductive systems that result in these aquatic systems being net consumers of DOC, where bacterial respiration exceeds primary production. It is known that in coastal waters bacteria often process organic carbon which originated mainly inland (Opsahl & Benner 1997) and can certainly be biologically available (Carlsson & Granéli 1993), while it is thought that little of the DOC from open oceans is of terrestrial origin (Meyers-Schulte & Hedges 1986).

Since autotrophs have to cope with diel variations in light availability, it is assumed that more intense bacterial activities during light hours would indicate more intense coupling between the producers and the consumers (i.e. Fuhrman et al. 1985). Diel changes in bacterial production have been found in open ocean samples (Burney et al. 1982, Johnson et al. 1983), in slope waters (Fuhrman et al. 1985), and in coastal waters (Sieburth et al. 1977, Meyer-Reil et al. 1979, Hagström & Larsson 1984). In other studies in coastal waters, however, some authors have not found consistent diel variations (Riemann & Søndergaard 1984, Riemann et al. 1984).

Our working hypothesis is that if bacteria are tightly coupled to variations in primary production, we should find a conspicuous diel cycle in bacterial growth with peaks of activity around noon. If bacteria are largely independent of DOC released from primary production, either because they use allochthonous carbon or because they depend on the degradation of POC, bacterial heterotrophic activity should not show such a pattern. One corollary of this hypothesis is that a more tightly coupled community should be found in the open ocean and a less tightly coupled situation should be found where DOC was available from sources other than primary production. The coupling between the production of newly formed DOC by algae and its use by bacteria depends, among other things, on how fast bacteria react to changes in the environment. It is an assumption of our hypothesis that if bacteria respond slowly, changes in growth may appear to be independent of changes in primary production at the sampled temporal scales. If changes are fast, and bacteria are tightly coupled to the producers, we should find changes in bacterial activity throughout the diel cycle.

Data on bacterial activity from oligotrophic open ocean systems are scarce, and even more so for the Mediterranean Sea. In spite of the general oligotrophy of this sea, however, the presence of mesoscale features is known to generate enhancements of primary production (Estrada 1996). We were interested in determining the degree of coupling between primary and bacterial producers in such an oligotrophic ocean. Thus, we sampled a gradient of inshore-offshore influence in the Catalan Sea, NW Mediterranean, and performed day and night samplings in 5 stations in 3 sites. The first site was located on the continental shelf (depth <70 m) where we expected to find higher coastal runoff influence and elevated surface chlorophyll levels; the second site was on the shelf slope (depth ~1000 m), subject to sporadic bursts of primary production; and the third site was in the open sea (depth 2100 m), where we could expect a clear stratification with, perhaps, increasing levels of plankton production in the pycnocline. We expected to see marked diel cycles of bacterial activity offshore and no cycles or very weak cycles inshore. We sampled the stations several times a day during at least 2 diel cycles and measured several microbial parameters as well as the vertical profiles of DOC.

MATERIAL AND METHODS

We studied an inshore-offshore transect in the NW Mediterranean Sea starting from the city of Barcelona between June 23 and 29 (1993) and between June 6 and 24 (1995) on board BIO 'Hespérides' (Fig. 1). In 1993 we studied 1 diel cycle at 2 stations (W and E). In 1995 we selected 3 stations on the same transect and we sampled each one at 4 h intervals during two 24 h periods, i.e. 6 profiles were measured during each 24 h period. The selected stations were on the continental shelf (Stn C, for coastal); on the shelf slope (Stn S, for slope) and in the salinity dome (see below) several miles offshore and in the deepest area (Stn D, for deep). Both the transect and the stations selected are standard study sites in this area of the NW Mediterranean where research into other components of the food web has been performed in the past (Calbet et al. 1996, Estrada 1996, C. Pedrós-Alió, J. I. Calderón-Paz, N. Guixa-Boixereu, M. Estrada & J. M. Gasol unpubl.). Salinity, fluorescence, temperature, oxygen and pressure data were obtained with a Neil Brown Mark III CTD probe. Water samples were taken with a 12 Niskin bottle rosette attached to the CTD. Samples for bacterial measurements were kept in polyethylene bottles in an opaque plastic box with surface water until used for the activity measurements. Incubations were started less than 20 min after water collection.

DOC. Water samples for DOC measurements were collected in polyethylene bottles that had previously been soaked in diluted sodium hypochlorite and hydrochloric acid and then cleaned with Milli-Q water. Just before filling them, the bottles were rinsed 3 times with the sampled water. Filtration was done on Whatman GF/F filters and a glass filtration system that had been rinsed as the bottles above. DOC analyses were performed within a few days of collection in a Shimadzu TOC-5000 operating with the High Temperature Catalytic Oxidation (HTCO) method. Samples were acidified with 0.5 ml of 2.5 N HCl to pH 2. Intense bubbling of pure synthetic air through the sample for 10 min allowed complete decarbonation. A glass syringe was used to pump the decarbonated sample through a combustion quartz tube filled with a 0.5% Pt on Al₂O₃ catalyst. A total of 3 to 5 replicate injections were done per sample. DOC concentration was obtained by subtracting instrument blank area from peak area and dividing by the slope of the standard curve as in Thomas et al. (1995). The instrument blank is the system blank plus the filtration blank. The system blank was determined by subtracting the DOC in UV-Milli-Q from the total blank. Measurements made with the high sensitivity catalyst (Pt on silica wool) produced values <2 µM C for fresh UV-Milli-Q water. The filtration blank was determined by filtering UV-Milli-Q water through the filtration system and was ~5 µM C. Before sample analysis, the catalyst was washed by injecting UV-Milli-Q for at least 12 h until the system blank was low and stable. The system blank was <8 µM C. The system was standardized with potassium hydrogen phthalate (KHP). The coefficient of variation of the peak area for the 3 to 5 replicates of each sample was ~1%. We participated in the international intercalibration exercise conducted by J. Sharp (Univ. of Delaware) obtaining satisfactory results (within ±10%; J. Sharp pers. comm.).

Bacteria. Samples for bacterial abundance were fixed with formaldehyde (3% final) or with glutaraldehyde (1% final) immediately after collection. A few hours after fixation, 10 to 20 ml was filtered onto 0.2 µm black polycarbonate filters and stained with DAPI (4',6-diamidino-2-phenylindole) for 5 min. The filters were then mounted with low-fluorescence oil on microscope slides and stored frozen until they were counted by epifluorescence microscopy in a Nikon Diaphot microscope. About 300 bacteria were counted per sample. Bacterial size was determined after measurement of approximately 200 cells with an image analysis system attached to the microscope. We custom-modified the software NIH-Image to size bacteria.

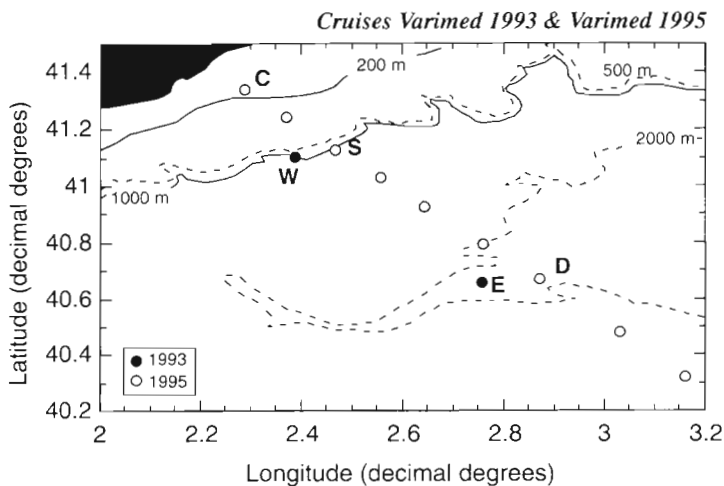


Fig. 1. Map of the area studied during cruises Varimed 1993 and Varimed 1995. The transect of 1995 follows a line between the city of Barcelona and the channel between Menorca and Mallorca. The stations where diel cycles were studied are marked with the letters W (West) and E (East) in 1993, and C (coastal), S (slope) and D (deep) in 1995

The characteristics of the system, the calibration with latex beads, and the choice of filters to process the images are detailed in Massana et al. (1997). The choice of formulas to compute volume from the 2-dimensional measurements was based on Fry (1990) and is described in the above-mentioned work. Since the Mediterranean is an oligotrophic sea, bacteria-sized prochlorophytes could be mistaken for bacteria and significantly modify the estimates of specific activity (see e.g. Sieracki et al. 1995). In June 1996 we were able to analyze samples from the same area and stations by flow cytometry. Prochlorophytes were found at maximum abundances of $1 \times 10^4 \text{ ml}^{-1}$, equivalent to <5% of the DAPI counts (authors' unpubl. data) at this time of the year.

Bacterial biomass was calculated using the carbon to volume relationship derived by Norland (1993) from the data of Simon & Azam (1989):

$$\text{pg C cell}^{-1} = 0.12 \times (\mu\text{m}^3 \text{ cell}^{-1})^{0.7}$$

Nucleoid-containing cells (NUCC) were determined after Zweifel & Hagström (1995). Samples were killed with sodium azide (0.5 M final), diluted with 0.2 µm filtered Milli-Q water and then incubated for 2 h with DAPI (2 µg ml⁻¹ of sample) and Triton X-100 (0.1 v/v). They were then collected onto the polycarbonate filters. Ten ml 2-propanol for destaining of the DAPI was then filtered, and the filter was air-dried and mounted. These samples were viewed with a Zeiss Axioplan epifluorescence microscope at ×1250 with filter set 450-490 FT 510 LP 520.

Bacterial activity. We estimated bacterial activity from both radioactive leucine and thymidine incorpo-

ration. For leucine, we used the method of Kirchman et al. (1985) as described in Kirchman (1993) with slight modifications. We added 20 nM leucine to triplicate vials. That was the saturating concentration found in 5 concentration-dependent incorporation experiments performed during the cruise. The commercial leucine solution was brought to 1 μ M and mixed with non radioactive leucine at 10% hot:90% cold. We used 20 ml vials as the incubation bottles. One of each was immediately killed with 4% formaldehyde (final). Vials were incubated in the dark at temperatures as close as possible to *in situ* by using either running surface water or water baths adjusted to the necessary temperatures. Incubations lasted 120 to 150 min in accordance with results of time course experiments. After incubation, the samples were killed with formaldehyde, filtered through 0.22 μ m cellulose acetate filters, and rinsed twice in 5% ice-cold TCA (trichloroacetic acid) and 3 times with 80% ethanol. The filters were dissolved with 0.5 ml of ethyl acetate and 4.5 ml of Opti-phase Hisafe II scintillation cocktail was added before counting, on board, with a Beckman scintillation counter.

For bacterial production measured using the ^3H -thymidine incorporation method, we used the Fuhrman & Azam (1980) method as modified by Smith & Azam (1992). Samples were incubated with 10 nM ^3H -thymidine (final concentration).

Conversion factors used. We carried out the exercise of calculating bacterial production only for comparison to DOC variations throughout the diel cycle. We applied several different conversion factors (CF): for leucine, we used a standard CF of 1.15×10^{17} cells mol^{-1} (average of all open ocean factors according to Kirchman et al. 1995), and the empirical factors calculated for this area, which were 2.1 kg C mol^{-1} for the coastal station, 1.5 kg C mol^{-1} for the slope station and 0.3 kg C mol^{-1} for the deep ocean station (Pedrós-Alió et al. unpubl.). This latter value, although low, is close to the factor found by Carlson et al. (1996) for the Sargasso Sea. For thymidine uptake, we used a standard factor of 2×10^{18} cells mol^{-1} (Bell 1993) as well as an empirical one of 5.9×10^{17} cells mol^{-1} also calculated for this area (Pedrós-Alió et al. unpubl.). We think that these theoretical and empirical values bracket the best probable estimates of bacterial production calculated from the activity data.

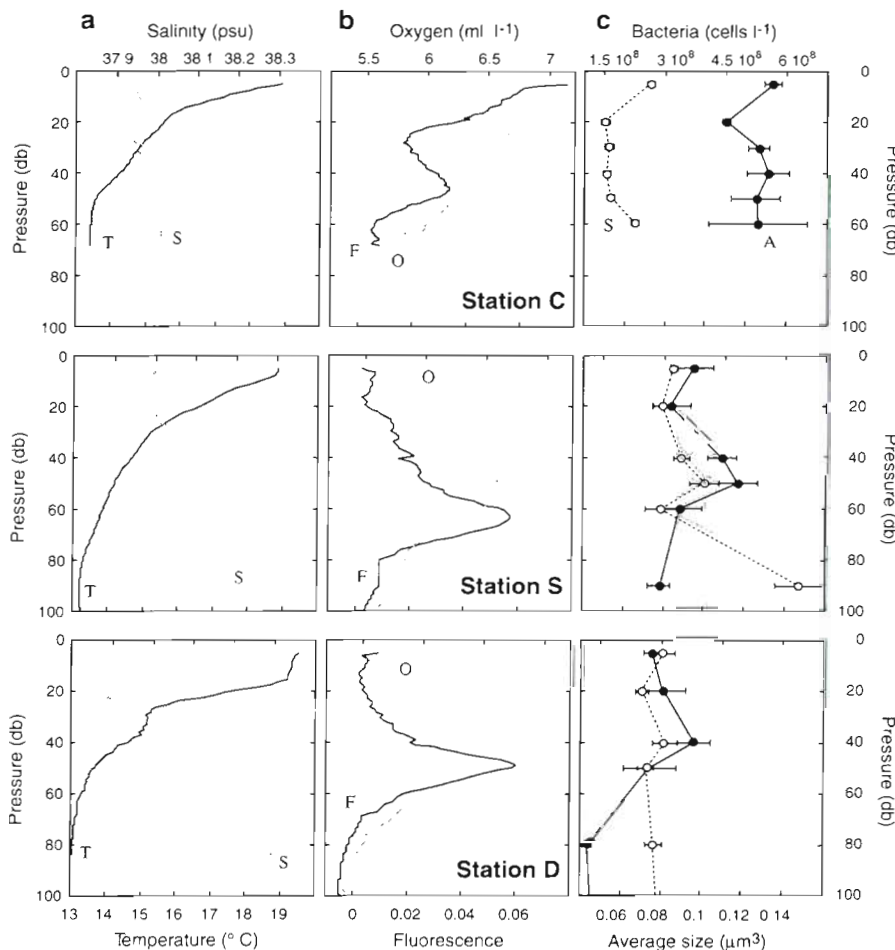


Fig. 2. (a) CTD profiles of temperature and salinity in the 3 stations during 1995. (b) CTD profiles of oxygen and fluorescence for the same stations. These profiles correspond to the initial sampling for the first diel cycle followed in each station. (c) Some representative parameters of the bacterial assemblage in the 3 stations: diel-averaged bacterial abundance (\pm SE, \bullet) and average size for the first samples of the day (\pm SE, \circ)

Depth-averaging and integration. We depth-averaged the data following the integration method C as described in Massana & Pedrós-Alió (1994). Briefly, this involves delimiting intervals with the midpoints of the 2 consecutive sampling depths, and assuming the concentration in the interval to be that of the sampling depth in its center. The depths of averaging were 60 m for Stn C and 80 m for Stns D and S. Integrated values are produced as depth-averaged value times averaging depth.

RESULTS

Water mass characteristics during both June 1993 and June 1995 were usual for this time of the year in the NW Mediterranean. Low salinity coastal waters are found over the continental shelf and are separated from higher salinity waters by a shelf/slope front which acts as a boundary for coastal waters which do not seem to penetrate the open sea areas except through sporadic mesoscale circulation features (Salat 1996). A clear salinity dome could be seen at around 60 km from the coast that, by the shallowing of the nutricline, can sporadically increase primary production (e.g. Falkowski et al. 1991, Estrada 1996). A deep chlorophyll maximum (DCM) detected as a deep fluorescence at around 50 m was generally found in all of the stations offshore of the continental shelf (see examples in 1995, Fig. 2). The highest chlorophyll concentrations at the DCM level were generally found in the vicinity of the frontal structures. In coastal waters, high fluorescence values are also found at the surface (Salat 1996, and Fig. 2).

During 1993 we studied one station on the slope (Stn W) and another one in the open Mediterranean far away from the coastal influence but above the salinity dome (Stn E). In 1995, 2 stations similar to the ones studied in 1993 were analyzed and also 1 coastal station was included (Fig. 1). The stations studied in 1995 were all thermally stratified and presented a DCM (Fig. 2). Stn S showed a less marked pycnocline development than Stn D, and Stn C had a less marked DCM than those of the 2 other stations. Increased oxygen levels in the upper half of the DCM at all 3 stations (Fig. 2) suggest that those were the areas of higher primary productivity. At the 3 stations, bacterial abundance (DAPI-stained particles) showed slight maxima at the depth between the DCM and the oxygen maximum. Stn C had higher bacterial abundance than Stn S and the latter more than Stn D. However, the bacterial cells at Stn S were on average slightly larger ($0.09 \mu\text{m}^3$) than those at Stn D ($0.075 \mu\text{m}^3$) and a lot larger than those at Stn C ($0.055 \mu\text{m}^3$; Fig. 2).

Table 1. Depth-averaged total and specific bacterial activities (leucine incorporation) in Stns E and W during 1993 and in Stns C, S and D during 1995 at different times of the day. Also presented are the percentages of the daily mean value that could be measured at each time

Stn	Time (h)	Total activity (pmol Leu $\text{l}^{-1} \text{h}^{-1}$)	% of mean	Specific activity ($10^{21} \text{mol Leu cell}^{-1} \text{h}^{-1}$)	% of mean
W	07:15	39.48	97	2.78	94
W	15:10	46.86	115	3.22	109
W	22:37	37.28	92	2.79	95
W	02:07	39.41	97	3.01	102
E	07:49	56.76	100	2.89	98
E	13:10	69.72	123	3.60	122
E	21:00	56.91	100	3.11	106
E	02:37	43.37	77	2.15	73
C1	05:30	36.03	91	1.84	96
C1	10:23	57.16	144	2.70	141
C1	13:22	42.55	107	1.75	91
C1	17:10	26.23	66	1.09	57
C1	22:20	32.83	83	1.39	73
C1	03:13	43.14	109	2.69	141
C2	05:00	23.19	146	1.01	166
C2	09:58	10.04	63	0.38	63
C2	14:34	7.55	47	0.34	56
C2	17:11	15.58	98	0.53	87
C2	21:30	26.72	168	0.89	147
C2	03:12	12.42	78	0.50	82
S1	06:39	37.71	111	2.85	131
S1	12:30	43.13	127	2.72	125
S1	15:38	25.56	75	1.87	86
S1	21:53	46.31	137	2.95	136
S1	00:55	18.20	54	1.12	52
S1	03:36	32.53	96	1.51	70
S2	07:17	25.57	68	1.87	53
S2	09:37	33.12	88	2.78	79
S2	15:25	56.55	150	6.11	174
S2	18:27	26.87	71	2.80	80
S2	21:49	29.90	80	2.90	83
S2	00:40	53.53	142	4.63	132
D1	05:10	22.87	60	1.41	33
D1	11:50	31.69	83	3.91	91
D1	14:42	71.35	187	10.08	234
D1	17:22	35.65	94	3.79	88
D1	22:00	19.00	50	2.09	49
D1	01:25	47.84	126	4.52	105
D2	05:10	22.33	128		
D2	11:12	8.02	46	1.16	69
D2	15:10	39.97	229	3.54	210
D2	17:30	10.70	61	1.38	82
D2	21:33	13.71	79	1.24	74
D2	01:20	9.93	57	1.11	66

Diel cycles of bacterial activity

In the stations sampled in 1993, bacterial heterotrophic activity showed a clear diel pattern with higher integrated values around noon and lower values at night (Table 1). This diel pattern was similar in both stations (Fig. 3) and especially marked in the upper part of the DCM.

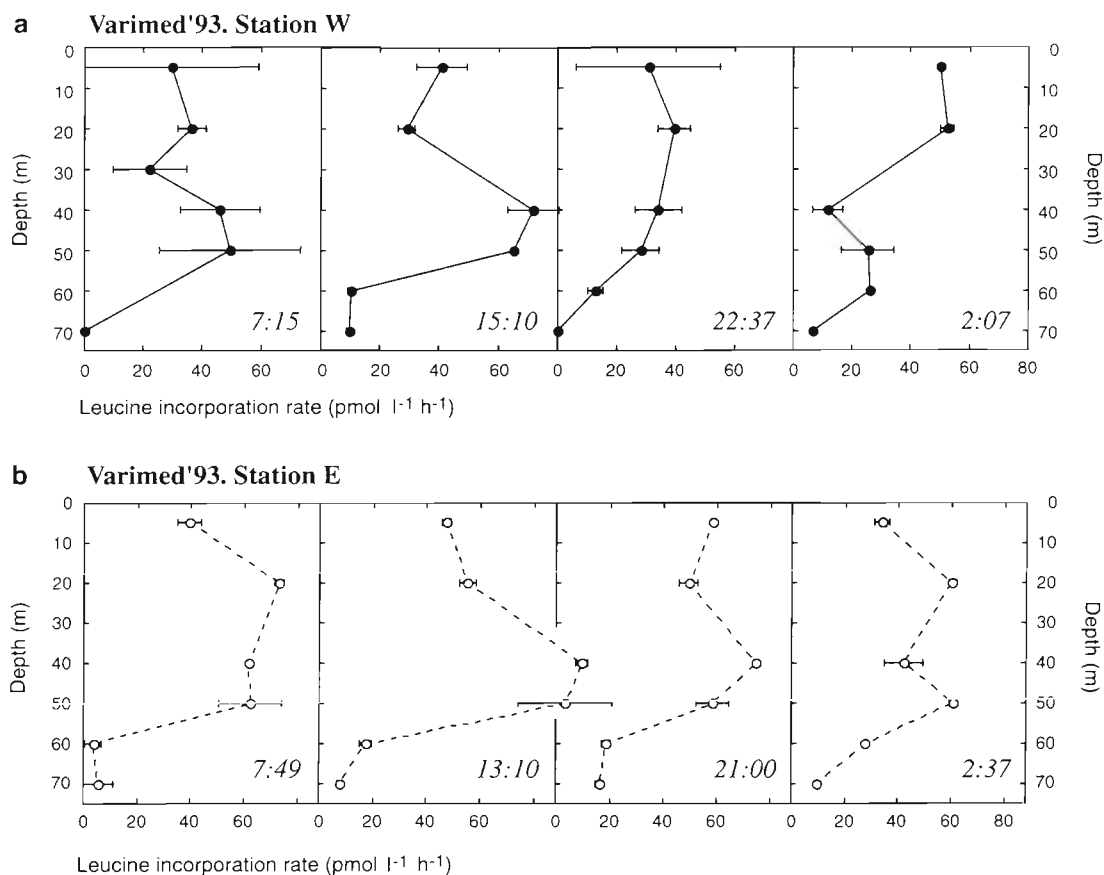


Fig. 3. Diel changes in bacterial activity during 1993 at (a) Stn W (●) and (b) Stn E (○). The values are averages and standard errors of 2 replicated measurements. The GMT time of each sampling is presented in each panel

In 1995, diel cycles were analyzed in more detail: 3 stations were studied twice and 6 profiles were measured along each diel cycle. The patterns of diel variation in Stn C were different during both sampling periods, and activity was in general lower in the second cycle (Fig. 4). Maxima of activity and of specific activity were detected at the surface in the early morning of the first cycle, and lower values were found throughout the rest of the day. During the second visit, total activity was highest at 20 to 30 m in the afternoon. In Stn S, during the first cycle, there were maximum activities at the surface during the morning and afternoon and at 100 m at noon (Fig. 5). Specific activities were at a maximum at the surface and at depth but lower between 20 and 50 m. During the second cycle, June 8 to 9, total activity was higher in the evening and at the surface and also at noon at 60 m (DCM). Altogether, specific activity was higher at noon and in the early afternoon than during the rest of the diel cycle. Stn D showed quite marked diel cycles both for total activity and for specific activity (Fig. 6). During the first visit, maximum activities were measured at the DCM around noon and at the surface in the afternoon. Spe-

cific activities were also higher at noon. During the second visit, the maximum total and specific activities detected at noon were specially marked at 20 m. We separately analyzed the different depths to see if there were general patterns in diel cycles at some depths that were not apparent at other depths. As for 1993 (Fig. 3), diel changes at the DCM were more apparent and more consistent than changes at other depths, and they followed a pattern almost identical to that presented in Fig. 7 and Table 1 for the depth-averaged values.

During 1 cycle at each station we also measured the diel changes in thymidine incorporation (Fig. 8). In Stn C there was a maximum of activity in the morning that remained only at the surface through the rest of the cycle. In Stns S and D, the pattern was similar: maximum values at the surface in the morning and at the DCM at around noon.

Depth-averaged (or integrated) values were used to provide an estimation of the diel changes that average the differences in the vertical profiles (Fig. 7, Tables 1 & 2). There was no consistent diel pattern for bacterial total or specific activity in Stn C. However, in both diel

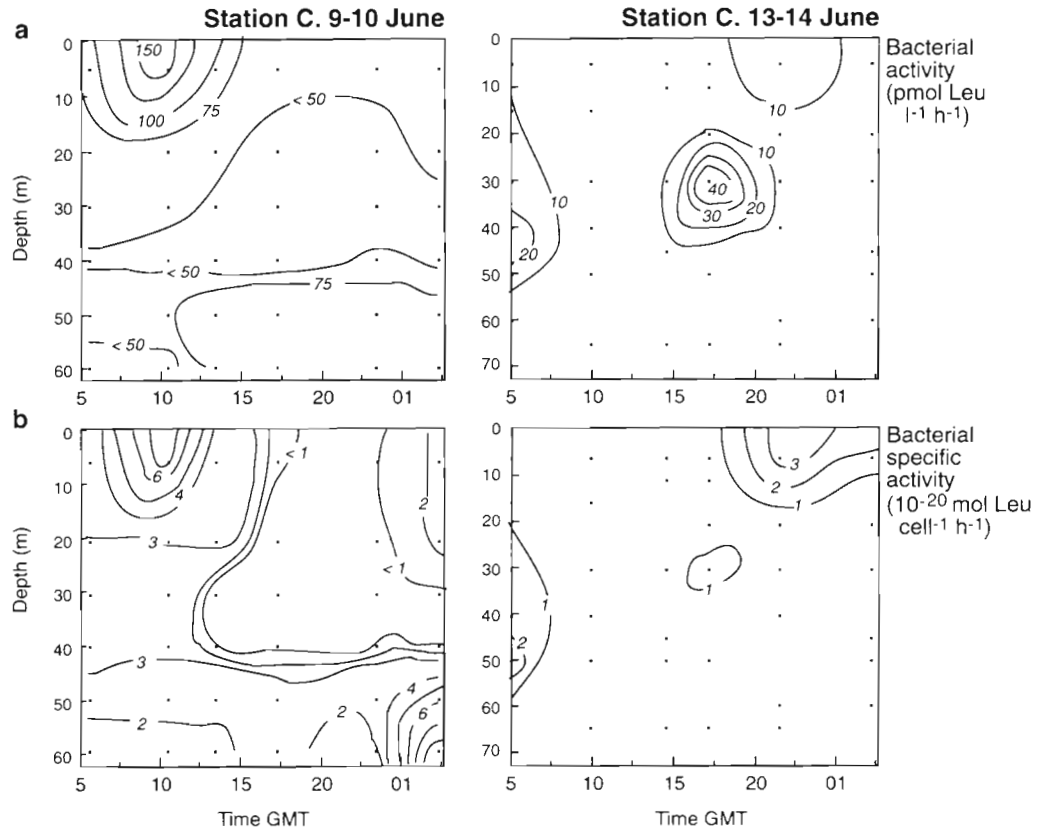


Fig. 4. Diel changes in total leucine incorporation rates (a) and specific incorporation rates (b) during the diel cycles at Stn C in 1995

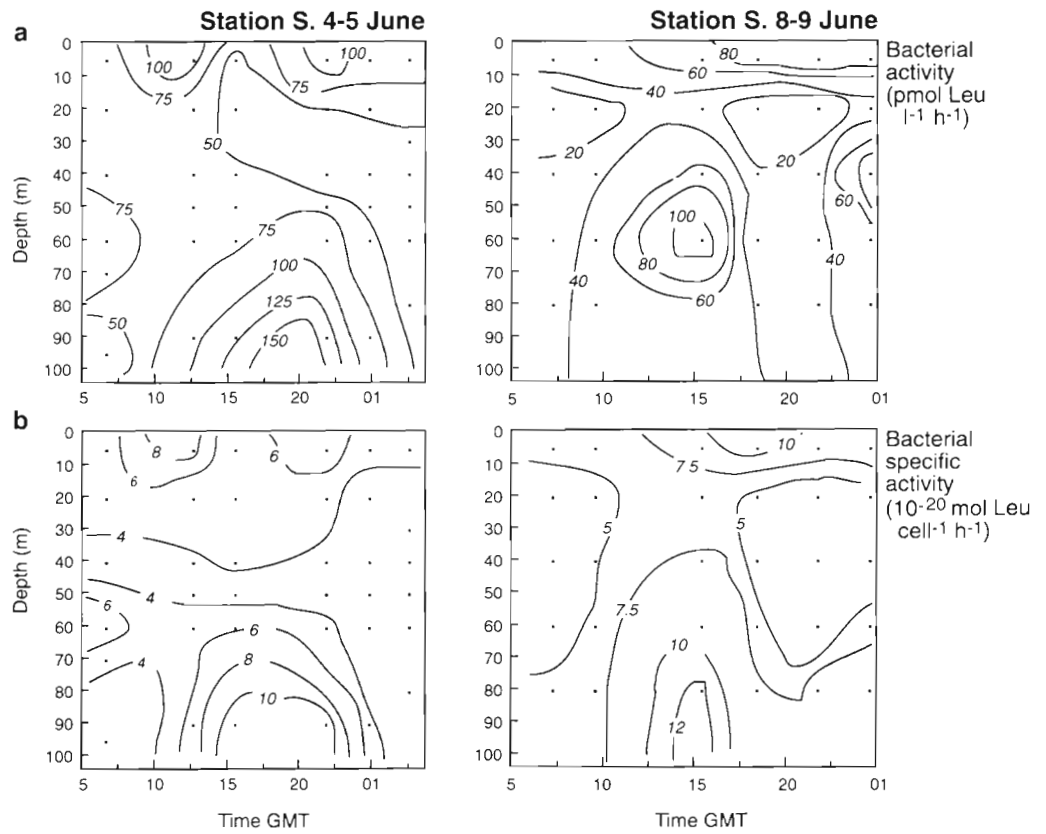


Fig. 5. Diel changes in total leucine incorporation rates (a) and specific incorporation rates (b) during the diel cycles at Stn S in 1995

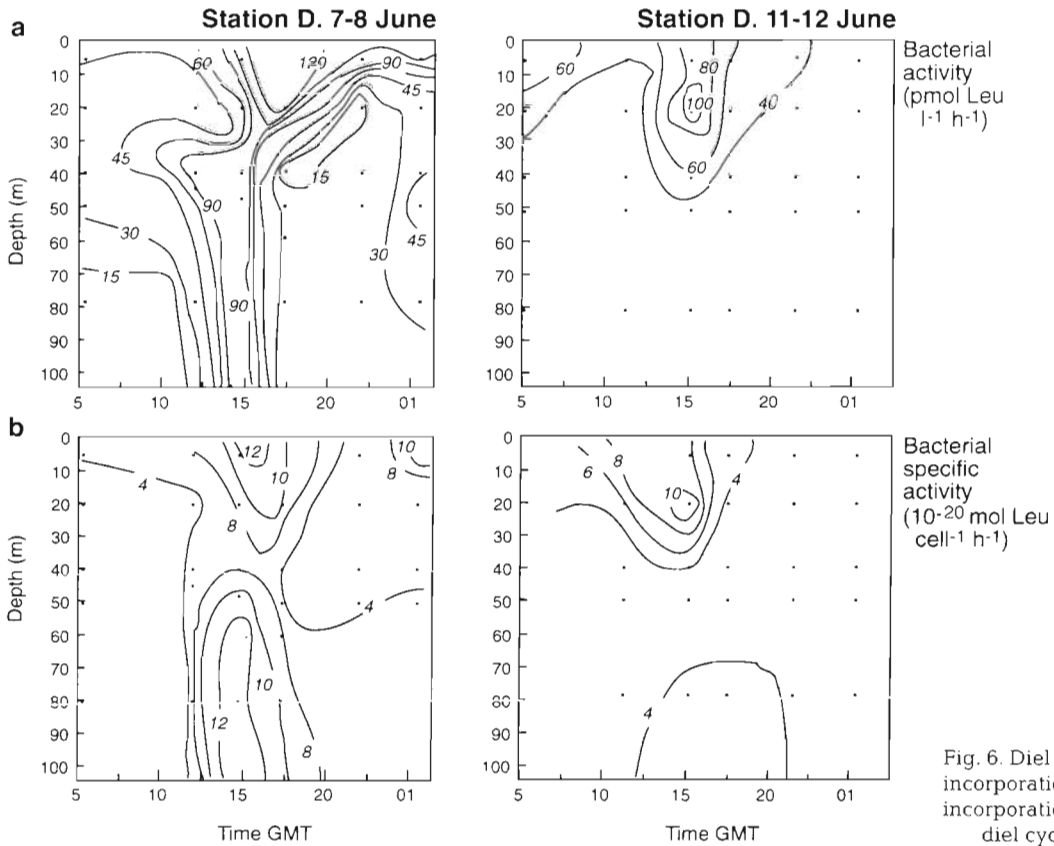


Fig. 6. Diel changes in total leucine incorporation rates (a) and specific incorporation rates (b) during the diel cycles at Stn D in 1995

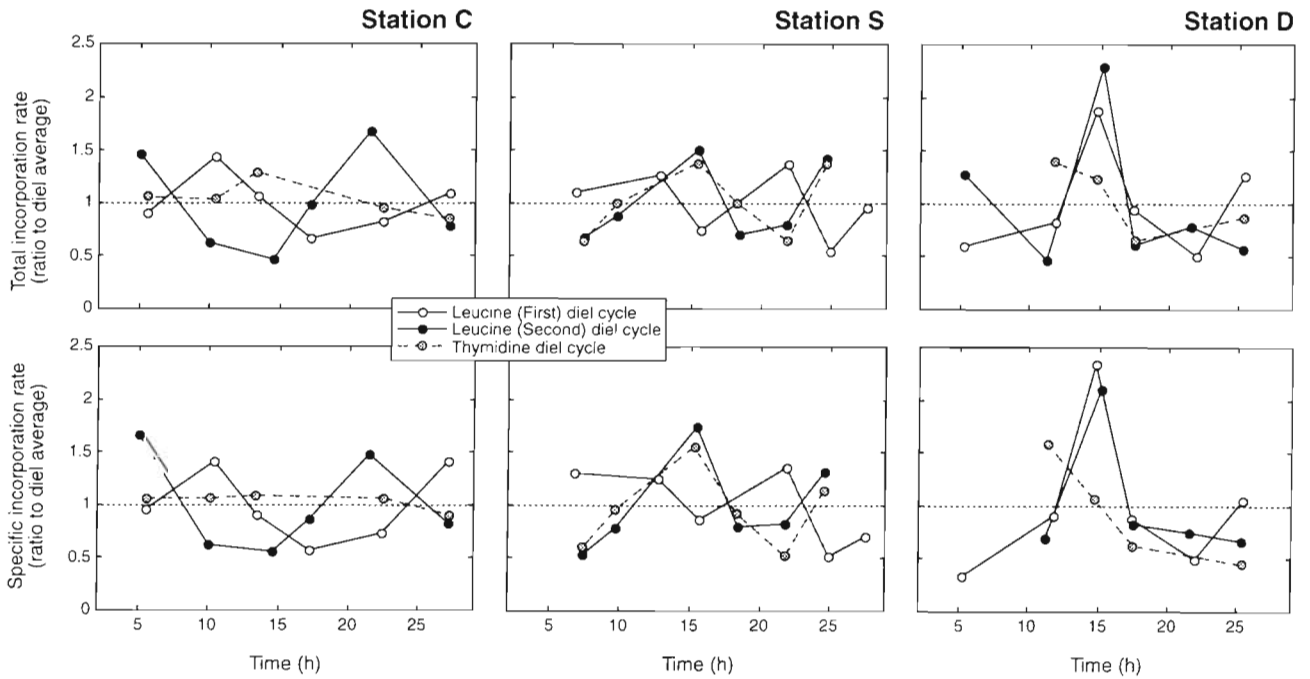


Fig. 7. Diel changes in total incorporation rates and specific incorporation rates of leucine (●, first diel cycle and ○, second diel cycle) and thymidine (◻) for the different diel cycles studied in each station in 1995, expressed as ratio to the diel average value (average value being 1)

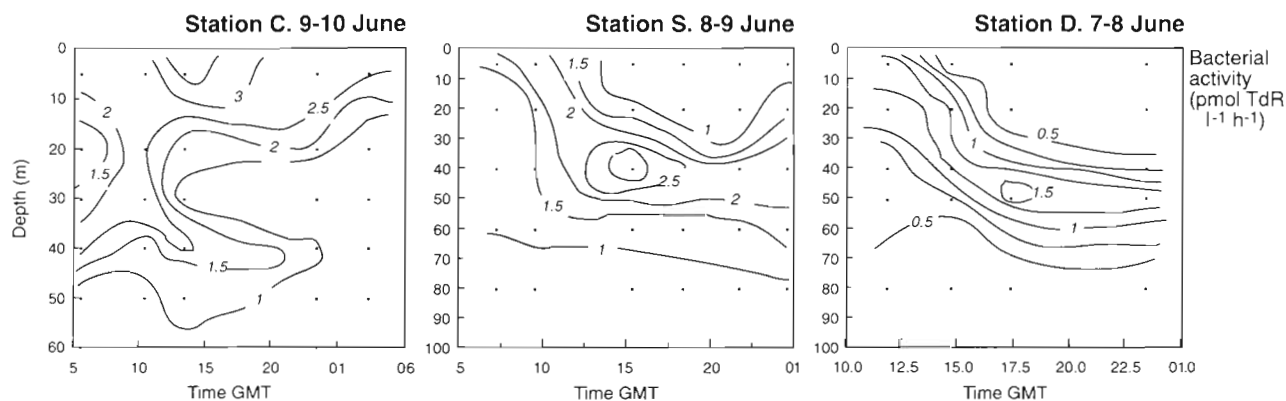


Fig. 8. Diel changes in total thymidine incorporation rates during a diel cycle in Stns C, S and D in 1995

cycles in Stn D, the values at noon were higher than those at all other times of the day. In Stn S, there was 1 noon value higher than the others, and 1 inconsistent pattern. At Stn C, the values of bacterial total or specific activity were always within the range 0.5 to 1.5 times the diel average. However, at Stn D, the values at noon were more than twice the diel average estimates, and the values in the afternoon and at night were less than half the diel average (Fig. 7). There was, thus, a >4-fold difference between some of the estimates of integrated bacterial activity, depending on the time of sampling.

Integrated thymidine uptake rates presented a pattern similar to that of leucine uptake in Stns S and D. Total and specific activity showed maximum values in the afternoon for Stn S and before noon for Stn D, although we missed the morning sampling in Stn D (Fig. 7, Table 2). The variations of depth-averaged total and specific activity throughout the diel cycle in Stn C were very low. The patterns of thymidine uptake in Stn S were almost identical to those of leucine uptake in Stn S, but seemed to be displaced in time at Stn D.

DOC diel cycle

The vertical profiles of DOC concentration in 1995 were significantly different from day to night (Fig. 9; non parametric paired sign test H_0 : no differences, $p < 0.0005$). They were higher at almost all depths at noon, and lower at night. In Stn C the maximum difference was found at 30 m, but in Stns S and D maximum differences between day and night were found at 20 m. At some depths the differences between day and night amounted to 26 μM , but on average they were 6 or 7 μM in Stns C and S, and ~ 10 μM in Stn D. Changes in total integrated DOC were quite significant throughout the diel cycle, ranging from 0.32 mol C m^{-2} (Stn S) to 0.85 mol C m^{-2} (Stn D). These diel changes in total integrated DOC represent between 5 and 15% of the

total DOC. This is the amount of DOC being produced and consumed throughout the diel cycle.

NUCC diel cycle

Between 30 and 50% of the DAPI-stained bacterial cells were found to contain nucleoids in 1995 (Fig. 10). There were more NUCC in Stn C at noon than at midnight. At Stns S and D, it was the other way around. The percentage of NUCC cells was quite similar during day and night in Stn C, but diel dynamics were discernible in the other 2 stations. It is interesting to note that the pattern of daily variation with depth was very similar in both Stns S and D: at surface and at the DCM there were more NUCC cells during the day than during the night. Above and below the DCM, however,

Table 2. Depth-averaged total and specific bacterial activities (thymidine incorporation) in Stns C, S and D during 1995 at different times of the day. Also presented are the percentages of the daily mean value that could be measured at each time

Stn	Time (h)	Total activity (pmol TdR $\text{l}^{-1} \text{h}^{-1}$)	% of mean	Specific activity (10^{-21} mol TdR $\text{cell}^{-1} \text{h}^{-1}$)	% of mean
C1	05:30	1.44	101	6.87	104
C1	10:23	1.44	101	7.20	109
C1	13:22	1.72	122	7.08	107
C1	22:20	1.37	97	6.87	104
C1	03:13	1.11	78	5.12	77
S2	07:17	0.50	62	3.32	48
S2	09:37	0.82	102	6.79	98
S2	15:25	1.10	136	11.75	169
S2	18:27	0.80	100	6.22	89
S2	21:49	0.52	65	4.76	69
S2	00:40	1.10	136	8.87	128
D1	11:50	0.81	130	11.52	161
D1	14:42	0.70	113	7.58	106
D1	17:22	0.42	68	5.23	73
D1	01:25	0.56	90	4.26	60

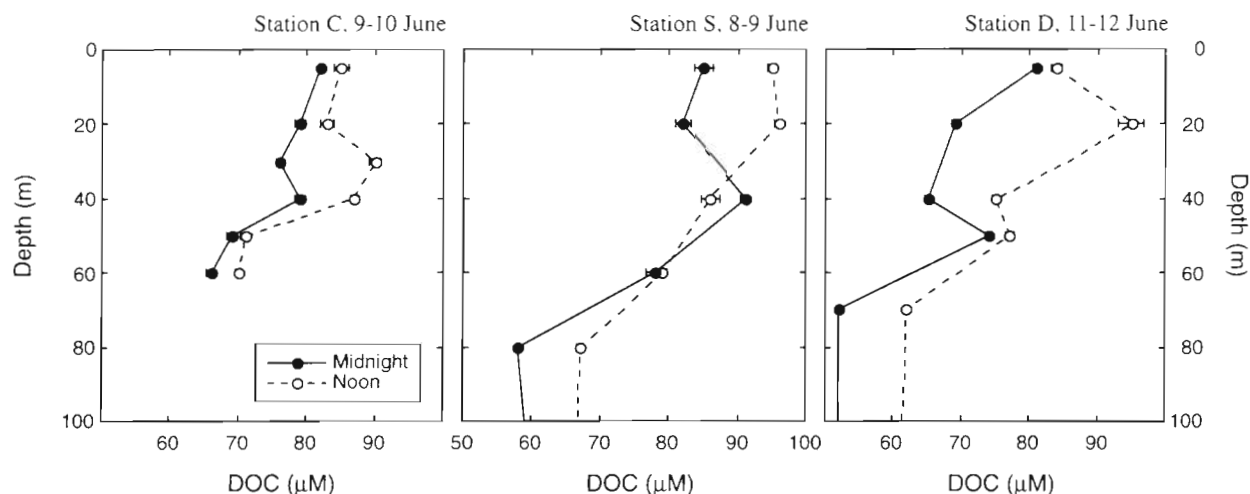


Fig. 9. Noon (O) and midnight (●) profiles of DOC concentration in the 3 stations in 1995. Error bars (sometimes hidden by the point) are the standard error of 3 to 5 replicates

the opposite was true. At Stn D we also measured NUCC at 05:00 h when lowest values were found over the diel cycle at all depths.

DISCUSSION

Marked diel cycles in total and specific bacterial heterotrophic activity were detected in some of the stations sampled. The diel changes were found both in DNA and in protein synthesis rates. These diel cycles were accompanied by changes in the distribution of total DOC, and in the proportion of bacteria containing visible nucleoids.

One of the problems of following the diel cycles of any community in the sea is that different water masses are sampled over time. Among all the strategies reviewed by Fuhrman et al. (1985), we decided to stay permanently in the same position. We integrated down to 60 (Stn C)–80 m the values of temperature and salinity for each of the CTD profiles from which we took samples to detect significant ship movements out of the initial water mass. We plotted these values in a temperature-salinity plot in which each point corresponds to the depth-average of each CTD cast (Fig. 11). Stn C, which had warmer temperatures and lower salinities, had signatures in this plot that did not change much during the diel cycle. Stn D showed higher salinities and intermediate temperatures. The effect of the diel warming of the water was quite apparent, but the 2 stations were quite similar. Stn S, with lower temperatures and intermediate salinities, behaved differently in the 2 visits. In the second one there were clear changes in water masses, moving from a slope situation to an open ocean one by the end of the diel cycle. We concluded from these graphs that, with the exception of Stn S dur-

ing June 8 and 9, we were quite probably sampling the same water mass during the whole day.

Diel changes in bacterial activities

Because, as will be shown later, bacterial abundance changed sometimes during the diel cycle, we looked at the diel changes not only of bacterial activity, but also of specific bacterial activity. We would be able to speak about consistent diel cycles of bacterial activity only if they were reflected by changing specific bacterial activities.

The thymidine uptake activities measured in this study ($0.5 \text{ pmol TdR l}^{-1} \text{ h}^{-1}$) were close to those measured in other Mediterranean areas (Zohary & Robarts 1992, Fernández et al. 1994, Robarts et al. 1996) and to those measured in other oligotrophic seas such as the Sargasso Sea (Carlson et al. 1996). There are no published data on leucine incorporation rates in the open Mediterranean, other than our own and our values of 10 to 120 $\text{pmol Leu l}^{-1} \text{ h}^{-1}$ are in the range of those encountered by Carlson et al. (1996) in the Sargasso Sea.

In Stn D bacterial activities followed a clear diel cycle (Figs. 6 & 8). Total activity changed from $<60 \text{ pmol l}^{-1} \text{ h}^{-1}$ in the early morning to values of $90 \text{ pmol l}^{-1} \text{ h}^{-1}$ around noon and back to lower values during the evening and night (Fig. 6). The diel pattern was even more obvious in integrated values (Fig. 7). In 1993, maximum bacterial activities were also measured around noon, especially at the depth of the DCM. The diel cycle was not as obvious from the TdR incorporation data (Fig. 8) except when depth-integrated (Fig. 7). Zweifel et al. (1993) and Simon (1994) also reported more marked diel cycles in leucine than in thymidine incorporation rates.

In Stn S, we found different patterns in the different visits. In 1993, a pattern close to that of Stn D was found. In 1995 we could not sample the same water mass during one of the cycles (Fig. 11) although we found some tendency for higher values of total and specific leucine and thymidine uptake activity around noon in one of the visits to the station. In the coastal station, however, changes in thymidine and leucine total and specific incorporation activity did not show any regular pattern.

Daily maxima of bacterial thymidine or leucine incorporation rates have been recorded in several marine locations, some coastal (Sieburth et al. 1977, Meyer-Reil et al. 1979, Moriarty & Pollard 1982, Fuhrman et al. 1985, Herndl & Malačić 1987, Wikner et al. 1990, Zweifel et al. 1993) and some oceanic (Sieburth et al. 1977, Burney et al. 1982, Johnson et al. 1983, Wheeler et al. 1989). There is also a report of minimum values at noon and higher values during the rest of the day (Zohary & Robarts 1992) for the Eastern Mediterranean, although these authors also measured extremely high diel bacterial and protozoan changes and their results are, thus, difficult to interpret.

Diel maxima of uptake of organic compounds have also been described in the open ocean. Carbohydrates (Sieburth et al. 1977, Burney et al. 1982, Johnson et al. 1983), amino acids (Carlucci et al. 1984), adenine (Winn & Karl 1986) or nitrogen uptake rates (Wheeler et al. 1989) have been shown to be higher during the day in oceanic stations. In some cases, evening or night maxima in frequency of dividing cells (Hagström & Larsson 1984) and thymidine incorporation rates (Paul et al. 1988, Simon 1994, Torréton et al. 1994, Jeffrey et al. 1996) have been related to night time stimulation of bacterial production as a consequence of diel zooplankton migration.

Since both nutrient availability and bacterivory contribute to determine the levels of bacterial biomass and activity, we should consider whether changes in nutrients and/or in predation pressure caused the changes in bacterial heterotrophic activity during the diel cycle. Obviously, primary production is higher when light is present and the amount of excreted photosynthate probably is also (Hama et al. 1988). The role of predation is not that clear: lower grazing rates at night have been suggested (Fuhrman et al. 1985, Weisse 1989) or

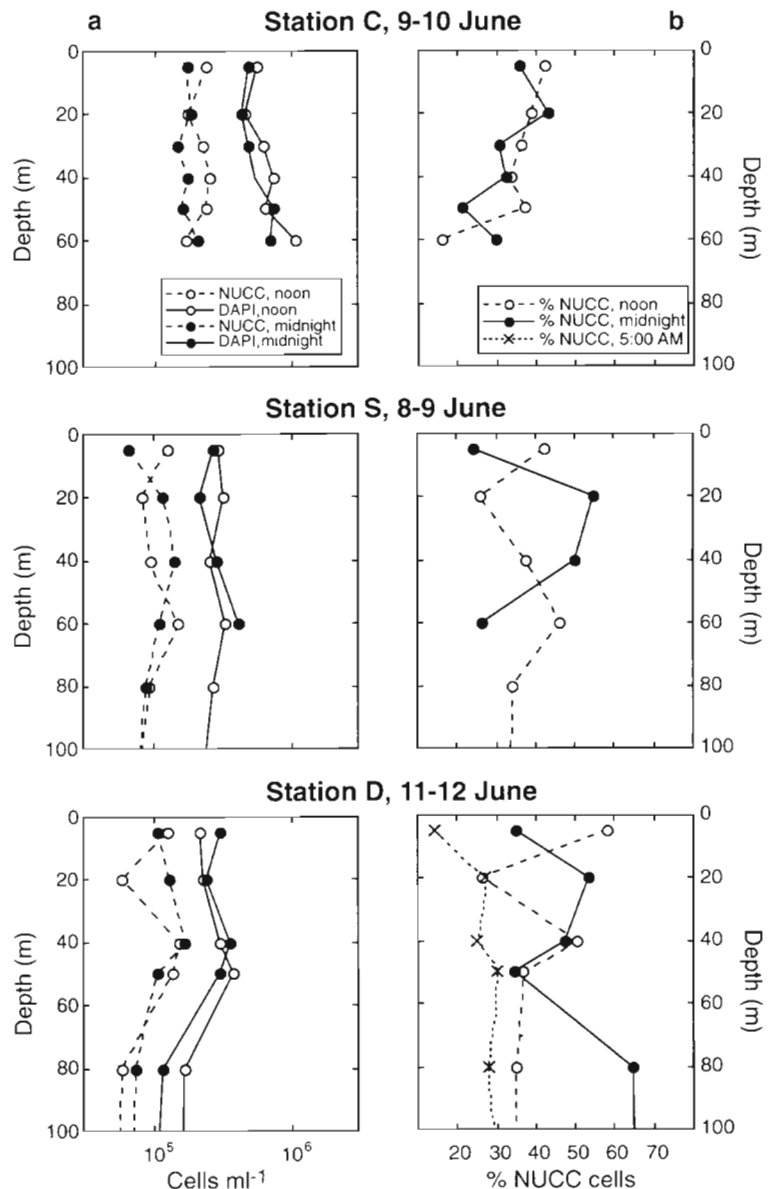


Fig. 10. (a) Noon (○) and midnight (●) profiles of DAPI-stained bacterial cells and nucleoid-containing bacterial cells in the 3 studied stations in 1995. (b) Noon (○) and midnight (●) profiles of nucleoid-containing cells as a percentage of the total DAPI-stained assemblage. Note that in Stn D, we also took samples at 05:00 h (×)

demonstrated (Wikner et al. 1990) to occur, while no diel cycle in viral abundance has been found in the reports we are aware of (Jiang & Paul 1994, authors' unpubl. data).

It is interesting to point out that a lack of diel patterns in bacterial activity has only been reported for coastal areas (Riemann & Søndergaard 1984, Turley & Lochte 1986, Gocke et al. 1987) or eutrophic lakes (Marcussen et al. 1984), although non significant results may not always be reported.

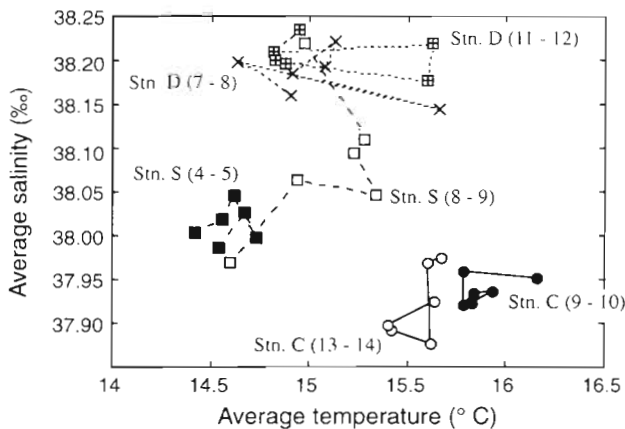


Fig. 11 Depth-averaged values of temperature and salinity and their changes during the diel cycles in Stn C (●, first and ○, second cycle), Stn S (■, first and □, second cycle) and Stn D (×, first and ⊠, second cycle). The numbers in the figure refer to the dates of sampling

There is a tendency for diel changes in bacterial activity to be well marked in oligotrophic and/or open ocean areas, and to be less common or less marked in coastal stations. Zweifel et al. (1993) compared results of mesocosms performed in the Baltic and in the Mediterranean and found more clear diel cycles in the Mediterranean than in the Baltic because of stable weather conditions and pronounced day/night cycles in the Mediterranean. We would now add, on the basis of our results and the above literature review, that the oligotrophic nature of the Mediterranean and the lower impact of land-originated materials in this sea could help explain the differences with the Baltic.

As stated by Fuhrman et al. (1985), tight coupling between phytoplankton and bacteria would require DOC sources for bacterial growth closely linked to primary production, because as more steps are added between carbon fixation and bacteria, more lags and less coupling should be expected. Thus, our results would strongly argue for well coupled phytoplankton-bacteria relationships in the most oceanic stations and uncoupled relationships in the coastal station where bacterial growth would be very dependent on allochthonous substrates. Moreover, we have also shown by averaging data from several years that the ratio of bacterial production to primary production is much higher in Stn C (average 77%; Pedrós-Alió et al. unpubl.) than in Stn D (average 10%) with low values again similar to the recent estimates for the Sargasso Sea (Carlson et al. 1996). The biomass ratios were also high for Stn C (bacterial biomass 114% of algal biomass), and lower in Stn D (50%), data that would support our arguments for relevant allochthonous carbon subsidies to the bacterioplankton in Stn C and a tighter relationship between bacteria and phytoplankton in Stn D.

Consequences for the routine estimation of bacterial production

One consequence of the patterns in diel variability in bacterial activity that we have shown is that the estimates of bacterial activity in open ocean samples will depend on the time of the day in which they are taken (Tables 1 & 2). If our results for open ocean samples are confirmed and following the same line of reasoning that proposed specific protocols for the measurement of primary production (like the JGOFS protocols), we should recommend the establishment of a general protocol for the estimation of bacterial production. This protocol would include sampling at least at noon and at night when intending to perform carbon balance measurements including bacterial production.

Diel cycle of nucleoid-containing bacteria

The relatively large shifts in NUCC numbers found throughout the diel cycle (Fig. 10) are interesting in several ways. First, changes were certainly more important at Stns S and D (7 to 10% night-day difference) than at Stn C (3% difference) and in the one station where an additional sampling had been done in the early morning the values were then much lower (water-column average 27% NUCC versus 40% at noon and 45% at midnight). The almost negligible changes in Stn C would be consistent with the hypothesis that bacteria in this station are uncoupled from the daily variations of primary production. The higher changes in the other stations would suggest that bacteria respond strongly to the diel changes in primary production, results again consistent with the diel variability in bacterial activity.

Second, the results would also advocate a small re-assessment of the meaning of the NUCC cells. Zweifel & Hagström (1995) suggested that the non-NUCC were probably empty dead bacterial cells. Other studies have suggested that some non-NUCC bacteria are capable of growth and become NUCC if the conditions are right (Choi et al. 1996). The latter authors also proposed the hypothesis that NUCC were those bacteria that had enough DNA (or in a condensed enough way) to precipitate DAPI. Perhaps a more feasible hypothesis could be that some cells are in some kind of resting (spore?) states with membrane conditions that do not let through the stain. They would thus show as non-NUCC. The higher proportion of NUCC cells that we found at night would perhaps indicate that some bacteria changed their membrane characteristics or synthesized much more DNA at night, which made them detectable as NUCC cells only at night. During the day, either because grazing selected them, or because

some cells returned to the resting states, we detected lower NUCC percentages. Further work on the short-term changes in NUCC numbers needs to be carried out to clarify the meaning of the data presented.

Diel changes in DOC concentration and the role of bacterial activity

The vertical profiles of DOC concentration were similar to profiles measured in other Mediterranean areas (Cauwet et al. 1990, Copin-Montégut & Avril 1993) and in other oligotrophic systems (Carlson & Ducklow 1995, Thomas et al. 1995, see 'Discussion' in Doval et al. in press). The daily changes in DOC in the 3 stations were important: around 15 μM (5 to 15% of the total stock) daily. This DOC should be highly labile since it appears and disappears daily. The fact that this daily variation could be detected, however, is an indication that the mechanism of production-consumption is not very well coupled (Carlson & Ducklow 1995). Some authors believe that this uncoupling is due to a possible nutrient (phosphorus) limitation of pelagic bacteria in the Mediterranean (Thingstad & Rassoulzadegan 1995). A study of DOC distribution in the NW Mediterranean estimated that ~20% of the DOC was labile (Doval et al. in press) based on the concentrations of DOC at lower depths. It would thus seem that most of the labile DOC had half-life times of only some hours.

The rate of daily appearance/disappearance was comparable to other values in the literature: Burney et al. (1981) found changes in the Sargasso Sea of around 20 to 40% daily. In the Caribbean, Burney et al. (1982) and Johnson et al. (1983) found changes of 2 to 15 $\mu\text{M d}^{-1}$. Kirchman et al. (1991) found 3 to 36% of the DOC being consumed in 24 h (average 25% in 2 d); Coffin et al. (1993) measured DOC turnover rates on the basis of oxygen consumption of 0.1 to 22% d^{-1} ; Zweifel et al. (1993) found that 7% of the DOC pool was being consumed daily in mesocosms; and Carlson & Ducklow (1996) found that 6 to 9% of the total DOC was being used in 2 d in Sargasso seawater cultures. The carbohydrate removal rate found by Burney (1986) in different communities enclosed within *in situ* containers ranged between 3.2 and 7.9 $\mu\text{g C l}^{-1} \text{h}^{-1}$, relatively close to the 15 μM in 12 h (15 $\mu\text{g C l}^{-1} \text{h}^{-1}$) that we found. Thus, the rates that we have extrapolated from our day and night measurements seem to be in the same range as other rates measured either *in situ*, in microcosms or in the laboratory.

Daily changes in biologically labile materials are commonly described: cAMP (Ammerman & Azam 1981), DOC and dissolved free amino acids (DFAA) and glucose uptake rates in the open Baltic Sea

(Meyer-Reil et al. 1979), dissolved carbohydrates (Sieburth et al. 1977, Burney et al. 1979) or DFAA (Mopper & Lindroth 1982) have been shown to change daily, and their diel changes assumed to be driven by heterotrophic activity. Mopper & Lindroth (1982) estimated that 3 to 6 $\mu\text{g DFAA l}^{-1} \text{h}^{-1}$ were being produced and consumed daily, a value that corresponded to 2 to 4 $\text{g C m}^{-2} \text{d}^{-1}$ (for a 60 m water column). A similar value (4 to 24 $\text{g C m}^{-2} \text{d}^{-1}$) was arrived at by Johnson et al. (1983) on the basis of the diel fluctuations in DOC in the Caribbean Sea. Our integrated values of DOC change throughout the diel cycle (4 to 10 $\text{g C m}^{-2} \text{d}^{-1}$) compare well with the reported values (Table 3).

Because we measured bacterial activity with 2 methods and throughout the diel cycle, it is tempting to compare the estimates of bacterial production obtained with each method to the daily disappearance of DOC. The actual values of bacterial production are dependent upon the choice of the conversion factor (Table 3) and the assumption that the carbon-to-volume conversion factors for bacteria are correct. We used empirical conversion factors that changed from station to station. The higher discrepancies were found in Stn D which was also the site where the empirical conversion factor was much lower than the theoretical (see 'Materials and methods'). In Stn D bacterial production varied between 20 and 360 $\text{mg C m}^{-2} \text{d}^{-1}$ depending on the method and factor used (Table 3). Changes in DOC were much larger (on the order of

Table 3. Daily balance of bacterial utilization of the dissolved organic carbon (DOC). Integrated bacterial production estimated from leucine incorporation rates with the factor 1.15×10^{17} cells mol^{-1} (Th; average of all open ocean factors according to Kirchman et al. 1995) or with the empirical (Emp) conversion factors for the NW Mediterranean as found by Pedrós-Alió et al. (unpubl.). Integrated bacterial production estimated from thymidine incorporation rates with the factor 2×10^{18} cells mol^{-1} (Th) or with the empirical conversion factor (Emp) for the NW Mediterranean as found by Pedrós-Alió et al. (unpubl.). Growth efficiency estimated as (bacterial production $\times 100$)/ ΔDOC . Note that for Stn D, the diel cycle used for thymidine uptake is different than that used for the DOC diel changes

Stn	Leucine ($\text{g C m}^{-2} \text{d}^{-1}$)		Thymidine ($\text{g C m}^{-2} \text{d}^{-1}$)		ΔDOC ($\text{g C m}^{-2} \text{d}^{-1}$)
	Emp	Th	Emp	Th	
C 1	0.12	0.18	0.18	0.31	4.17
C 2	0.05	0.07			
S 1	0.10	0.30			
S 2	0.11	0.34	0.20	0.33	3.84
D 1	0.02	0.36	0.13	0.22	
D 2	0.01	0.13			10.23
% efficiency					
C 1	2.89	4.34	4.34	7.36	
S 2	2.82	8.88	5.08	8.61	
D 1/2	0.10	1.23	1.29	2.19	

several $\text{g C m}^{-2} \text{d}^{-1}$) than the average bacterial production ($\sim 100 \text{ mg C m}^{-2} \text{d}^{-1}$).

The calculated efficiencies for bacterial growth (cell-based carbon growth efficiency = biomass production during the whole day/DOC change night-day) were, thus, very small: 2 to 8% in Stns C and S, and below 2% in Stn D (Table 3). These values are low, but close to those encountered in open ocean communities when computed with similar methods: 1.6 to 9% in the North Atlantic (Kirchman et al. 1991), 4 to 9% (Hansell et al. 1995) or 7 to 19% (Carlson & Ducklow 1996) for the Sargasso Sea. In coastal areas, values of 20% in an estuary (Bjørnsen 1986), 30 to 61% (Kroer 1993) and 27% (Zweifel et al. 1993) have been measured. As found by Biddanda et al. (1994), the carbon conversion efficiencies that we calculated were lower in Stn D than in those stations influenced by the coast. It appears that very low carbon conversion efficiencies are typical of open-ocean bacterial communities.

The rates of bacterial activity seem to suffice to explain the rates of disappearance of DOC from day to night, if we consider that the measured bacterial carbon conversion efficiencies are correct. Primary production rates in this area, however, amount to $0.5 \text{ g C m}^{-2} \text{d}^{-1}$ (Estrada 1996). Thus, where do these 4 to $10 \text{ g C m}^{-2} \text{d}^{-1}$ come from? This apparent paradox was also found by Burney et al. (1982) in the Caribbean Sea: the rates of diel carbohydrate concentration change required primary production values 10 times larger than those actually measured.

Labile DOC is assumed to originate from excretion of photosynthate; release by zooplankton activity (Peduzzi & Herndl 1992), dissolution of fecal pellets, cell lysis from viral infection (Fuhrman 1992), light-induced degradation of refractory DOC (Kieber et al. 1989, Wetzel et al. 1995), egestion by microzooplankton (Nagata & Kirchman 1992) or even production of DOC by bacteria through enzymatic breakdown of POC or from other sources (e.g. Iturriaga & Zsolnay 1981). We did not evaluate these sources of DOC but it seems unlikely that any one of them could explain the rates of daily accumulation of DOC presented in this work and in the literature. Excretion of low molecular weight photosynthate during primary production measurements during this study was less than 30% of primary production (Morán & Estrada pers. comm.). Zooplankton biomass in this area is at most 800 mg C m^{-2} and their ingestion rates have been estimated to be around 40 mg C m^{-2} (Calbet et al. 1996): even if all carbon ingested by zooplankton was excreted as DOC it would not explain the measured diel DOC change. Of all the factors that can contribute to the DOC pool, those that we can educately guess give estimates far from the observed values. We will have to wait until estimates of the other processes are available to solve this paradox.

Diel variability in the leucine versus thymidine uptake rates

Leu and TdR incorporation rates were not correlated even though the general pattern of activity throughout the diel cycle was quite similar (Tables 1 & 2). As noted above, diel variability of Leu incorporation was larger than the variations in TdR incorporation. Thymidine rates were clearly higher at the surface and at the DCM, and much lower below those layers (Fig. 12). However, we could not find any consistent trend for Leu incorporation rates with depth. These rates were much higher at noon than at any other time during the day, while the only significant general pattern for TdR uptake was the lower values in the afternoon (Fig. 12).

It has been suggested that the ratio between Leu and TdR incorporation rates should be constant if bacterial growth were balanced (Kirchman et al. 1985, 1986, Chin-Leo & Kirchman 1988). Large values of the Leu:TdR ratio would indicate biomass increases without concomitant DNA synthesis rates, and low values of the ratio would indicate DNA synthesis without biomass increase. Unbalanced growth may be produced by changes in dissolved organic matter (DOM) supply rates. Chin-Leo & Kirchman (1990) suggested that the diel variations in primary production and zooplankton grazing were likely to cause unbalanced growth in bacterial assemblages. Good correlation between rates of incorporation of both precursors have sometimes been found (Chin-Leo & Kirchman 1988, Kirchman & Hoch 1988, Chin-Leo & Benner 1992, Kirchman 1992), but large discrepancies are common in some environments (McDonough et al. 1986, Chin-Leo & Kirchman 1990). A tendency for relatively higher Leu:TdR ratios in richer environments (Servais 1991) or for uncorrelated (Yoshinaga et al. 1991) and very different values of Leu and TdR uptake in oligotrophic waters (Torréton & Dufour 1996) would point to differences in the source of the organic matter in each area.

We found lower values of the Leu:TdR ratio in the DCM, and very high values below these depths, which would indicate that bacteria were increasing their biomass but not dividing below the DCM (Fig. 12). Values at noon were much lower than those at other times of the day, which would perhaps indicate that at those times bacteria were simultaneously synthesizing protein and DNA, while mainly protein was synthesized at other times of the day. Diel variability in the Leu:TdR ratio was found by Kirchman et al. (1986) in waters off the coast of Florida. Leu incorporation occurred before TdR incorporation even though both maxima occurred in the late afternoon. Kirchman & Hoch (1988) in the Delaware Bay estuary found a peak in the Leu:TdR ratio after several hours of growth and thereafter the ratio declined. Chin-Leo & Benner (1991), studying a

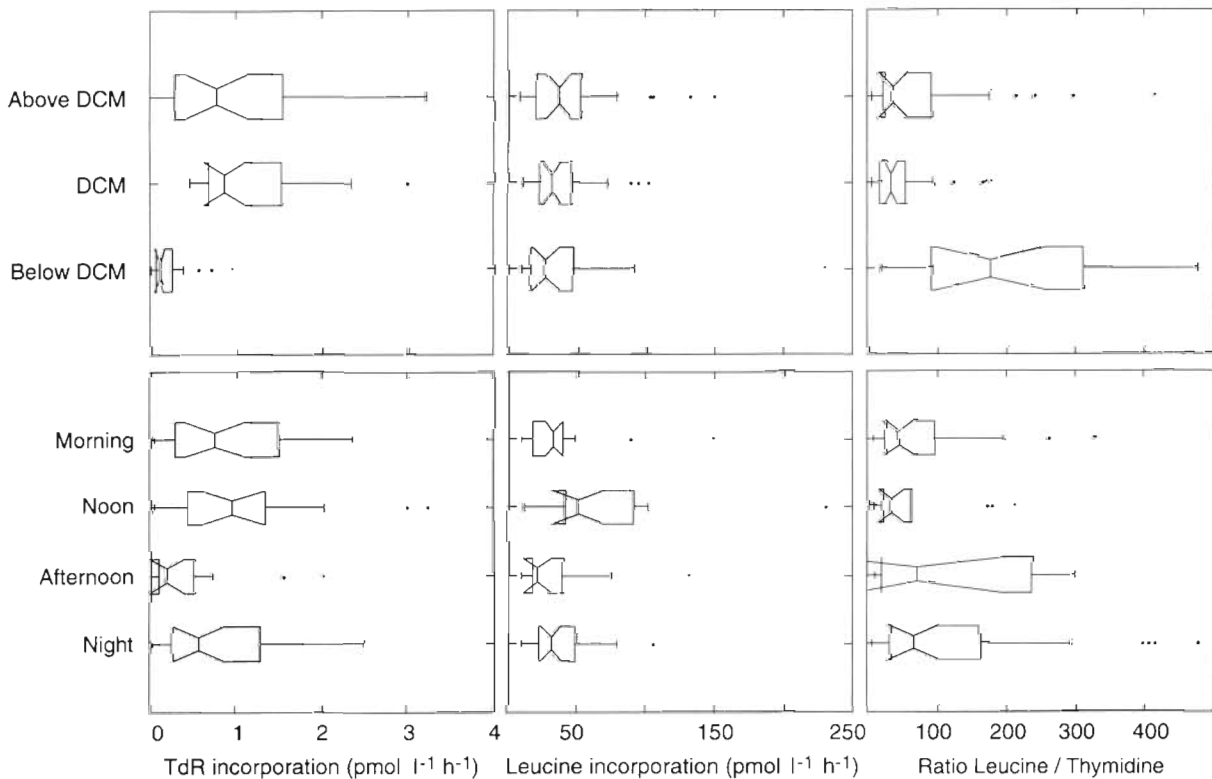


Fig. 12. Notch box-and-whisker plots of thymidine incorporation rates, leucine incorporation rates and the ratio of leucine to thymidine for different water layers (above, at, and below the DCM), and at different times of the day: morning, before 11:00 h; noon, between 11:00 and 17:00 h; afternoon, between 17:00 and 22:00 h; night, between 22:00 and 05:00 h

tropical lagoon, found low Leu:TdR ratios in the early morning and higher ratios in late evening, which would suggest a shift-up towards the evening. Our data (especially Fig. 8 compared to Figs. 4 to 6) also suggested that TdR incorporation increases before Leu uptake, which was concentrated in the noon hours, when we would expect the maximal amount of DOM to flow from photosynthate.

If the Leu:TdR ratio is taken as an indicator of balanced bacterial growth, it is clear that bacteria were growing better at the DCM layers and at noon. And it is quite probable then that the conversion factor required to convert Leu or TdR incorporation rates to bacterial production would be different in the DCM layers than above or below. The CF, therefore, should be determined every time if precise estimations of carbon flux through bacteria are required.

Acknowledgements. We thank everybody on board BIO 'Hespérides' for their help and good humor especially those who had to share the FH radioactive lab with us. We thank T Granata for providing the physical data; Å. Rudehäll for her skilful technical assistance; S. Canut for providing the usual guidance; G. García for measuring bacterial size; and C. Marasé and M. Estrada for helpful comments. This work was supported by grants AMB 94-1019 and 94-0853 from CICYT,

PB91-075 from DGICYT and MAS2-CT93-0063 and MAS3-CT95-0016 grants from the EU to the ICM and to Å. Hagström. M.D.D. was supported by a fellowship from the EU MAS2-CT93-0065 project. J.M.G. thanks the Max Planck Institute at Plön for providing the quiet needed to finish this paper.

LITERATURE CITED

- Ammerman JW, Azam F (1981) Dissolved cyclic adenosine monophosphate (cAMP) in the sea and uptake of cAMP by marine bacteria. *Mar Ecol Prog Ser* 5:85–89
- Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. *Mar Ecol Prog Ser* 10:257–263
- Azam F, Hodson RE (1977) Size distribution and activity of marine microheterotrophs. *Limnol Oceanogr* 22:492–501
- Bell RT (1993) Estimating production of heterotrophic bacteria via incorporation of tritiated thymidine. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) *Handbook of methods in aquatic microbial ecology*. Lewis Publishers, Boca Raton, p 505–508
- Biddanda B, Opsahl S, Benner R (1994) Plankton respiration and carbon flux through bacterioplankton on the Louisiana shelf. *Limnol Oceanogr* 39:1259–1275
- Björnson PK (1986) Bacterioplankton growth yield in continuous seawater cultures. *Mar Ecol Prog Ser* 30:191–196
- Burney CM (1986) Diel dissolved carbohydrate accumulation in coastal water of South Florida, Bermuda and Oahu. *Estuar Coast Shelf Sci* 23:197–203

- Burney CM, Davis PG, Johnson KM, Sieburth JMcN (1981) Dependence of dissolved carbohydrate concentrations upon small scale nanoplankton and bacterioplankton distributions in the western Sargasso Sea. *Mar Biol* 65:289–296
- Burney CM, Davis PG, Johnson KM, Sieburth JMcN (1982) Diel relationships of microbial trophic groups and in situ dissolved carbohydrate dynamics in the Caribbean Sea. *Mar Biol* 67:311–322
- Burney CM, Johnson KM, Lavoie DM, Sieburth JMcN (1979) Dissolved carbohydrate and microbial ATP in the North Atlantic: concentrations and interactions. *Deep Sea Res* 26:1267–1290
- Calbet A, Alcaraz M, Saiz E, Estrada M, Trepát I (1996) Planktonic herbivorous food webs in the Catalan Sea (NW Mediterranean): temporal variability and comparison of indices of phyto-zooplankton coupling based on state variables and rate processes. *J Plankton Res* 18:2329–2347
- Carlson CA, Ducklow HW (1995) Dissolved organic carbon in the upper ocean of the central equatorial Pacific Ocean, 1992: daily and finescale vertical variations. *Deep Sea Res* 42:639–656
- Carlson CA, Ducklow HW (1996) Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. *Aquat Microb Ecol* 10:69–85
- Carlson CA, Ducklow HW, Sleeter TD (1996) Stocks and dynamics of bacterioplankton in the northwestern Sargasso Sea. *Deep Sea Res* 43:491–515
- Carlsson P, Granéli E (1993) Availability of humic bound nitrogen for coastal phytoplankton. *Estuar Coast Shelf Sci* 36:433–447
- Carlucci AF, Shimp SL, Craven DB (1984) Diel production and microheterotrophic utilization of dissolved free amino acids in waters off Southern California. *Appl Environ Microbiol* 48:165–170
- Cauwet G, Sempere R, Salliot A (1990) Carbone organique dissous dans l'eau de mer: confirmation de la sous-estimation antérieure. *CR Acad Sci Paris* 311:1061–1066
- Chin-Leo G, Benner R (1991) Dynamics of bacterioplankton abundance and production in seagrass communities of a hypersaline lagoon. *Mar Ecol Prog Ser* 73:219–230
- Chin-Leo G, Benner R (1992) Enhanced bacterioplankton production and respiration at intermediate salinities in the Mississippi River plume. *Mar Ecol Prog Ser* 87:87–103
- Chin-Leo G, Kirchman DL (1988) Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine. *Appl Environ Microbiol* 54:1934–1939
- Chin-Leo G, Kirchman DL (1990) Unbalanced growth in natural assemblages of marine bacterioplankton. *Mar Ecol Prog Ser* 63:1–8
- Cho BC, Azam F (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature* 332:441–443
- Choi JW, Sherr EB, Sherr BF (1996) Relation between presence-absence of a visible nucleoid and metabolic activity in bacterioplankton cells. *Limnol Oceanogr* 41:1161–1168
- Coffin RB, Connolly JP, Harris PS (1993) Availability of dissolved organic carbon to bacterioplankton examined by oxygen utilization. *Mar Ecol Prog Ser* 101:9–22
- Cole JJ, Findlay S, Pace ML (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar Ecol Prog Ser* 43:1–10
- Copin-Montégut G, Avriil B (1993) Vertical distribution and temporal variation of dissolved organic carbon in the northwestern Mediterranean Sea. *Deep Sea Res* 40:1963–1972
- del Giorgio PA, Cole JJ, Cimleris A (1997) Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* 385:148–151
- Doval MD, Pérez FF, Berdalet E (in press) Dissolved and particulate organic carbon in the northwestern Mediterranean. *Deep Sea Res*
- Ducklow HW, Carlson CA (1992) Oceanic bacterial production. *Adv Microb Ecol* 12:113–181
- Estrada M (1996) Primary production in the northwestern Mediterranean. *Scient Mar* 60:55–64
- Falkowski PG, Ziemann D, Kolber Z, Bienfang PK (1991) Role of eddy pumping in enhancing primary production in the ocean. *Nature* 352:55–57
- Fernández M, Bianchi M, van Wambeke F (1994) Bacterial biomass, heterotrophic production and utilization of dissolved organic matter photosynthetically produced in the Almeria-Oran front. *J Mar Syst* 5:313–325
- Fry JC (1990) Direct methods and biomass estimation. *Methods Microbiol* 22:41–85
- Fuhrman JA (1992) Bacterioplankton roles in cycling of organic matter: the microbial food web. In: Falkowski PG, Woodhead AD (eds) Primary productivity and biogeochemical cycles in the sea. Plenum Press, New York, p 361–383
- Fuhrman JA, Azam F (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. *Appl Environ Microbiol* 39:1085–1095
- Fuhrman JA, Eppley RW, Hagström Å, Azam F (1985) Diel variations in bacterioplankton, phytoplankton, and related parameters in the Southern California Bight. *Mar Ecol Prog Ser* 27:9–20
- Gocke K, Kremling K, Osterroht C, Wenck A (1987) Short-term fluctuations of microbial and chemical variables during different seasons in coastal Baltic waters. *Mar Ecol Prog Ser* 40:137–144
- Hagström Å, Larsson U (1984) Diel and seasonal variations in growth rates of pelagic bacteria. In: Hobbie J, Williams PJ leB (eds) Heterotrophic activity in the sea. Plenum Press, New York, p 249–262
- Hama T, Matsunaga K, Handa N, Takahashi M (1988) Day-night changes in production of carbohydrate and protein by natural phytoplankton population from Lake Biwa, Japan. *J Plankton Res* 10:941–955
- Hansell DA, Bates NR, Gundersen K (1995) Mineralization of dissolved organic carbon in the Sargasso Sea. *Mar Chem* 51:201–212
- Herndl GJ, Malačić V (1987) Impact of the pycnocline layer on bacterioplankton: diel and spatial variations in microbial parameters in the stratified water column of the Gulf of Trieste (Northern Adriatic Sea). *Mar Ecol Prog Ser* 38:295–303
- Iturriaga R, Zsolnay A (1981) Transformation of some dissolved organic compounds by a natural heterotrophic population. *Mar Biol* 62:125–129
- Jeffrey WH, von Haven R, Hoch MP, Coffin RB (1996) Bacterioplankton RNA, DNA, protein content and relationships to rates of thymidine and leucine incorporation. *Aquat Microb Ecol* 10:87–95
- Jiang SC, Paul JH (1994) Seasonal and diel abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the marine environment. *Mar Ecol Prog Ser* 104:163–172
- Johnson KM, Davis PG, Sieburth JMcN (1983) Diel variations of TCO₂ in the upper layer of oceanic waters reflects microbial composition, variation and possibly methane cycling. *Mar Biol* 77:1–10
- Kieber DJ, McDaniel J, Mopper J (1989) Photochemical source of biological substrates in seawater: implications for carbon cycling. *Nature* 341:637–639

- Kirchman DL (1992) Incorporation of thymidine and leucine in the subarctic Pacific: application to estimating bacterial production. *Mar Ecol Prog Ser* 82:301–309
- Kirchman DL (1993) Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) *Handbook of methods in aquatic microbial ecology*. Lewis Publishers, Boca Raton, p 509–512
- Kirchman DL, Hoch MP (1988) Bacterial production in the Delaware Bay estuary estimated from thymidine and leucine incorporation rates. *Appl Environ Microbiol* 45:169–178
- Kirchman DL, K'nees E, Hodson R (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Appl Environ Microbiol* 49:599–607
- Kirchman DL, Murray RE, Hodson RE (1986) Rates of DNA and protein synthesis by heterotrophic bacteria in aquatic environments: a comparison between the thymidine and leucine approaches. *Proc IV ISME, Ljubljana, Slovenia*, p 631–637
- Kirchman DL, Rich JH, Barber RT (1995) Biomass and biomass production of heterotrophic bacteria along 140° W in the equatorial Pacific: effect of temperature on the microbial loop. *Deep Sea Res* 42:603–619
- Kirchman DL, Suzuki Y, Garside C, Ducklow HW (1991) High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. *Nature* 352:612–614
- Kroer N (1993) Bacterial growth efficiency on natural dissolved organic matter. *Limnol Oceanogr* 38:1282–1290
- Marcussen B, Nielsen P, Jeppesen M (1984) Diel changes in bacterial activity determined by means of microautoradiography. *Arch Hydrobiol Beih Ergeb Limnol* 19:141–149
- Massana R, Gasol JM, Bjørnsen PK, Blackburn N, Hagström A, Hietanen S, Hygum BH, Kuparinen J, Pedrós-Alió C (1997) Measurement of bacterial size via image analysis of epifluorescence preparations: description of an inexpensive system and solutions to some of the most common problems. *Scient Mar* 61:397–407
- Massana R, Pedrós-Alió C (1994) A method to determine integrated predation in stratified waters. *Limnol Oceanogr* 39:248–262
- McDonough RJ, Sanders RW, Porter KG, Kirchman DL (1986) Depth distribution of bacterial production in a stratified lake with an anoxic hypolimnion. *Appl Environ Microbiol* 52:992–1000
- Meyer-Reil LA, Bölter M, Liebezeit G, Schramm W (1979) Short-term variations in microbiological and chemical parameters. *Mar Ecol* 1:1–6
- Meyers-Schulte KJ, Hedges JI (1986) Molecular evidence for a terrestrial component of organic matter dissolved in ocean water. *Nature* 321:61–63
- Mopper K, Lindroth P (1982) Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis. *Limnol Oceanogr* 27:336–347
- Moriarty DJW, Pollard PC (1982) Diel variation of bacterial productivity in seagrass (*Zostera capricorni*) beds measured by rate of thymidine incorporation into DNA. *Mar Biol* 72:165–173
- Nagata T, Kirchman DL (1992) Release of dissolved organic matter by heterotrophic protozoa: implications for microbial food webs. *Arch Hydrobiol Beih Ergebn Limnol* 35:99–109
- Norland S (1993) The relationship between biomass and volume of bacteria. In: Kemp PF, Sherr BF, Sherr EF, Cole JJ (eds) *Handbook of methods in aquatic microbial ecology*. Lewis Publishers, Boca Raton, p 303–307
- Opsahl S, Benner R (1997) Distribution and cycling of terrigenous dissolved organic matter in the ocean. *Nature* 386:480–482
- Paul JH, DeFlaun MF, Jeffrey WH, David AW (1988) Seasonal and diel variability in dissolved DNA and in microbial biomass and activity in a subtropical estuary. *Appl Environ Microbiol* 54:718–727
- Peduzzi P, Herndl GJ (1992) Zooplankton activity fueling the microbial loop: differential growth response of bacteria from oligotrophic and eutrophic waters. *Limnol Oceanogr* 37:1087–1092
- Pomeroy LR (1974) The ocean's food web, a changing paradigm. *BioScience* 24:499–504
- Riemann B, Nielsen P, Jeppesen M, Marcussen B, Fuhrman JA (1984) Diel changes in bacterial biomass and growth rates in coastal environments, determined by means of thymidine incorporation into DNA, frequency of dividing cells (FDC), and microautoradiography. *Mar Ecol Prog Ser* 17:227–235
- Riemann B, Søndergaard M (1984) Measurements of diel rates of bacterial secondary production in aquatic environments. *Appl Environ Microbiol* 47:632–638
- Roberts RD, Zohary T, Waiser MJ, Yacobi YZ (1996) Bacterial abundance, biomass, and production in relation to phytoplankton biomass in the Levantine Basin of the southeastern Mediterranean Sea. *Mar Ecol Prog Ser* 137:273–281
- Salat J (1996) Review of hydrographic environmental factors that may influence anchovy habitats in northwestern Mediterranean. *Scient Mar* 60:21–32
- Servais P (1991) Bacterial production measured by ³H-thymidine and ³H-leucine incorporation in various aquatic environments. Abstracts 5th Int Workshop on the measurement of microbial activities in the carbon cycle of aquatic environments, Copenhagen
- Sieburth JMcN, Johnson KM, Burney CM, Lavoie DM (1977) Estimation of in situ rates of heterotrophy using diurnal changes in dissolved organic matter and growth rates of picoplankton in diffusion culture. *Helgoländer Wiss Meeresunters* 30:565–574
- Sieracki ME, Haugen EM, Cucci TL (1995) Overestimation of heterotrophic bacteria in the Sargasso Sea: direct evidence by flow and imaging cytometry. *Deep Sea Res* 42:1399–1409
- Simon M (1994) Diel variability of bacterioplankton biomass production and cell multiplication in Lake Constance. *Arch Hydrobiol* 130:283–302
- Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. *Mar Ecol Prog Ser* 51:201–213
- Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ³H-leucine. *Mar Microb Food Webs* 6:107–114
- Thingstad TF, Rassoulzadegan F (1995) Nutrient limitations, microbial food webs, and 'biological C-pumps': suggested interactions in a P-limited Mediterranean. *Mar Ecol Prog Ser* 117:299–306
- Thomas C, Cauwet G, Minster JM (1995) Dissolved organic carbon in the equatorial Atlantic Ocean. *Mar Chem* 49:155–169
- Torréon JP, Bouvy M, Arfi R (1994) Diel fluctuations of bacterial abundance and productivity in a shallow eutrophic tropical lagoon. *Arch Hydrobiol* 131:79–92
- Torréon JP, Dufour P (1996) Bacterioplankton production determined by DNA synthesis, protein synthesis, and frequency of dividing cells in Tuamotu Atoll lagoons and surrounding ocean. *Microbial Ecology* 32:185–202
- Turley CM, Lochte K (1986) Diel changes in the specific growth rate and mean cell volume of natural bacterial

- communities in two different water masses in the Irish Sea. *Microb Ecol* 12:271–282
- Weisse T (1989) The microbial loop in the Red Sea: dynamics of pelagic bacteria and heterotrophic nanoflagellates. *Mar Ecol Prog Ser* 55:241–250
- Wetzel RG, Hatcher PG, Bianchi TS (1995) Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnol Oceanogr* 40:1369–380
- Wheeler PA, Kirchner DL, Landry MR, Kokkinakis SA (1989) Diel periodicity in ammonium uptake and regeneration in the oceanic Subarctic Pacific: implications for interactions in microbial food webs. *Limnol Oceanogr* 34:1025–1033
- Wikner J, Rassoulzadegan F, Hagström Å (1990) Periodic bacterivory balances bacterial growth in the marine environment. *Limnol Oceanogr* 35:313–324
- Winn CD, Karl DM (1986) Diel nucleic acid synthesis and particulate DNA concentrations: conflicts with division rates by DNA accumulation. *Limnol Oceanogr* 31:637–645
- Yoshinaga I, Fukami K, Ishida Y (1991) Comparison of DNA and protein synthesis rates of bacterial assemblages between coral reef waters and pelagic waters in tropical ocean. *Mar Ecol Prog Ser* 76:167–174
- Zohary T, Roberts RD (1992) Bacterial numbers, bacterial production, and heterotrophic nanoplankton abundance in a warm core eddy in the Eastern Mediterranean. *Mar Ecol Prog Ser* 84:133–137
- Zweifel UL, Hagström Å (1995) Total counts of marine bacteria include a large fraction of non-nucleoid-containing bacteria (ghosts). *Appl Environ Microbiol* 61:2180–2185
- Zweifel UL, Norrman B, Hagström Å (1993) Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. *Mar Ecol Prog Ser* 101:23–32

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

*Submitted: August 21, 1997; Accepted: December 12, 1997
Proofs received from author(s): March 4, 1998*