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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)

Gerhard J. Herndl[1] & Vlado Malačič[2]

[1] Institute for Zoology, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria
[2] Marine Biology Station Piran, Cesta JLA 65, YU-66330 Piran, Yugoslavia

ABSTRACT: Diel variations in bacterial density, frequency of dividing cells (FDC) and dissolved organic carbon (DOC) in the stratified water column of the Gulf of Trieste were investigated at various depths. In the surface layers morning and afternoon DOC peaks (up to 10 mg l^{-1}) were observed in 3 out of 4 diel cycles. Bacterial abundance remained fairly constant over the diel cycles; however, bacterial activity as measured by FDC showed pronounced peaks in late afternoon and dawn coinciding with the DOC maximum concentrations. The pycnocline layer exhibited DOC concentrations similar to those of the overlying waters. The frequency of dividing cells (FDC), however, remained high throughout the entire diel cycle. The observed pronounced diel variations in bacterial production were therefore largely restricted to the layers well above the pycnocline; bacterial production contributed less than 20 % to the overall diel production (500 to 1000 μg C l^{-1} d^{-1}) in the uppermost 5 m water body. Highest diel production was found in the pycnocline layer at the end of the phytoplankton bloom (June and July) probably reflecting the formation of nutrient-enriched microzones around decaying phytoplankton cells due to reduced sinking velocities in the pycnocline layer. Additionally, FDC-based bacterial secondary production estimates were compared with *in situ* incubations of dialysis bags filled with 2 μm filtered seawater for 24 h. Bacterial biomass production estimates based on FDC for the 5 and 10 m layer were 3 and 14 times higher, respectively. The observed variations of microbial parameters within different layers point to the importance of small-scale investigations in both time and space.

INTRODUCTION

The tight coupling of pelagic primary production and bacterial utilization of phytoplankton extracellular release has been demonstrated by numerous authors (Itturiaga & Hoppe 1977, Wiebe & Smith 1977, Larsson & Hagström 1982, Bell 1983, Chrost 1983, Itturiaga & Zsolnay 1983). This coupling provokes measurable diel variations in biological and related parameters in the water column. Significant diel variations of bacterioplankton activity in response to varying photosynthetic rates have been reported from various marine environments (Sieburth et al. 1977, Meyer-Reil et al. 1979, Krambeck 1984, Riemann et al. 1984, Fuhrman et al. 1985, Turley & Lochte 1986). These studies clearly show high bacterial activity during daytime independent of the method used. Additional studies (Sieburth et al. 1977, Spencer 1979, Ammerman & Azam 1981, Burney et al. 1981) reveal a good agreement between bacterial activity and the amounts of various dissolved substances. Despite our knowledge on diel variations in bacterial biomass, its activity and related parameters, no attempt has been made – with the notable exception of Carlucci et al. (1986) – to investigate diel variations of several microbiological parameters in various layers of the water body concurrently.

Evidence has been presented in the literature that pronounced spatial variations in biological parameters occur within the water column due to diel activities of pelagic organisms. Nocturnal vertical migration and activity of zooplankton influences the content and composition of biological and chemical constituents in

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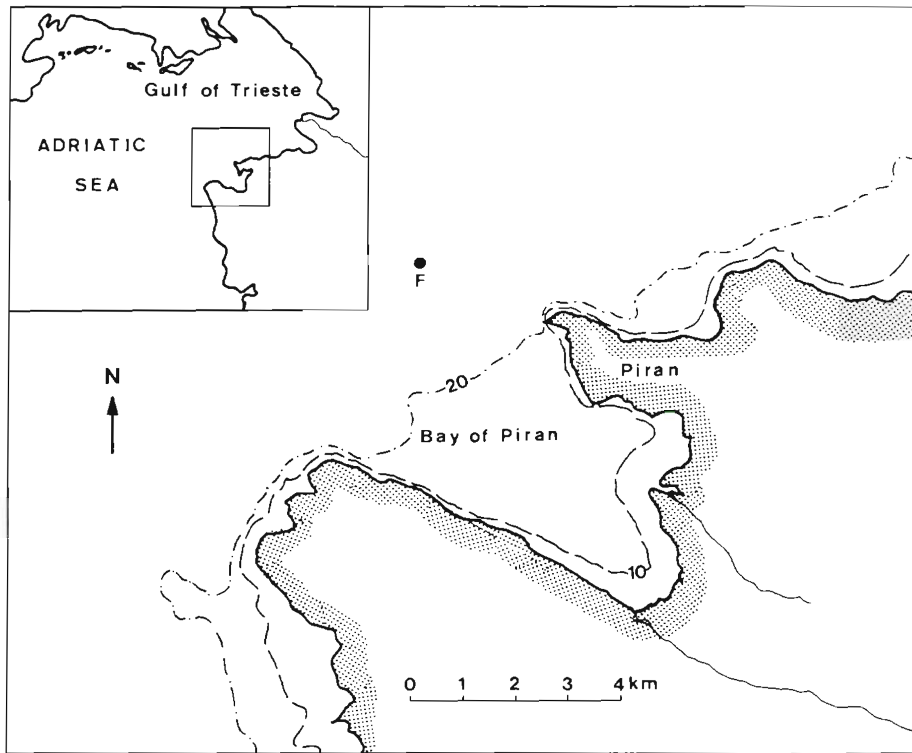


Fig. 1. Study area and position of sampling location (F)

near-surface layers (Lampert 1978, Copping & Lorenzen 1980, Eppley et al. 1981, Riemann et al. 1986) and may enhance bacterial activity (Jørgensen et al. 1983). On the other hand, diurnal phytoplankton primary production in surface waters is believed to cause an enrichment in dissolved organic matter (DOM) in these layers (Carlucci et al. 1984, 1985, 1986); this improves conditions for bacterial growth. Sieracki & Sieburth (1986), however, report on sunlight-induced growth delay of surface-layer bacteria incubated in bottles and submerged in outdoor aquaria. These authors conclude that direct or indirect photoinhibition of bacterial activities could be a significant factor in the diel cycling of organic matter in the euphotic zone (Sieracki & Sieburth 1986).

All the above-mentioned processes contribute to diel and spatial heterogeneity of the water column. Further discontinuity in the water column is introduced by stratification, leading to a reduced material flux between the different layers (Hargrave & Phillips 1986, Herndl et al. in press). As is true for other coastal embayments (Taft et al. 1980, Officer et al. 1984), the Gulf of Trieste (Northern Adriatic Sea) is characterized by strong stratification during summer. This may lead to hypoxia and occasionally to complete oxygen depletion of the near bottom layer (Stachowitsch 1984, Faganeli et al. 1985).

The purpose of this study is to determine the diel variations in bacterial density, bacterial activity and dissolved organic carbon in various depths of the stratified water column of the Gulf of Trieste. Sampling depths of each diel investigation period were adapted to the depth of the pycnocline; this enabled sampling in the close vicinity of this boundary layer since it was hypothesized that condensation of particulate organic matter (POM) in the pycnocline layer enhances bacterial activity. These studies of diel fluctuations will complement current investigations of seasonal aspects (Turk unpubl.). Specifically, it was asked whether diel fluctuations in microbial parameters necessitate a particular time of day for studying seasonal dynamics.

MATERIALS AND METHODS

Investigations were carried out on 24 to 25 Apr, 25 to 26 Jun, 28 to 29 Jul, and 22 to 23 Aug 1986, at the routinely sampled Stn F with a 22 m water column in the Gulf of Trieste (Northern Adriatic Sea) (Fig. 1).

A research vessel of the Marine Biology Station Piran was fixed at Position F by 3 anchors. For water sampling 5 l Niskin bottles were used, and the sampling depths varied according to the depth of the pycnocline. In June, bacterial secondary production was estimated using 2 µm filtered seawater from 5 and 10 m depth incubated in four 1 l dialysis bags (Reichert Thomapor 0.2 nm pore diameter) at the respective depths for 24 h (Turley & Lochte 1985, Herndl et al. in press).

At the beginning of each diel measurement, temperature was recorded continuously throughout the water column to determine the depth range of the pycnocline. Standard methods were used for biological and physical properties. Salinity was determined by the high precision titration method described in Strickland & Parsons (1972). Seawater for dissolved organic carbon (DOC) determination was gently vacuum-filtered through pre-rinsed 0.2 µm Sartorius filters and stored in precombusted glass vials (450 °C for 12 h) in the dark at 4 °C until analysis (generally within 14 d of sampling). DOC analyses were performed using a Beckman-Tocamaster 915-B. Calibration standards were made up from a stock solution (1000 mg C l^{-1}) of 2.125 g Reagent Grade anhydrous potassium biphthalate in 1 l of double-distilled water. Samples were freed of inorganic carbon by acidifying to about pH 2 with 50 µl HCl (conc. 2 %) and sparging with a stream of synthetic CO_2-free air. At least 2 replicates were made for each sample.

Water samples (10 ml) for bacterial abundance determination from both the water column and the dialysis bags were fixed with buffered formalin to a final concentration of 2 % and stored refrigerated. Bacterial density was determined within 14 d of sampling by acridine orange direct epifluorescence counts (AODC) (Hobbie et al. 1977). The same filters were used for determination of the frequency of dividing bacterial cells (FDC) (Hagström 1984). Bacterial cell dimensions were determined from visual estimates during AODC, in which 100 to 120 rods and 50 to 70 cocci were measured and the mean volumes calculated.

The precision of the methods used was tested on replicate estimates during the April sampling and is presented in Table 1 (DOC analyses were done routinely in duplicate).

Table 1. Coefficient of variation (CV) for replicate estimations on April sampling

Parameter	Average CV
Bacteria	0.3 %
FDC	4.3 %
DOC	1.9 %*

* Mean of all replicate determinations

RESULTS

The physical characteristics of the water column indicate that we sampled a well-stratified water body in all 4 diel cycles (Fig. 2). Fig 3 & 4 show the physical and microbiological parameters measured in April

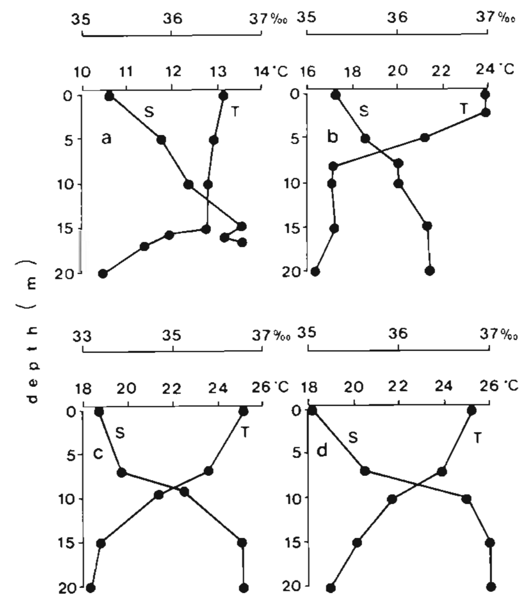


Fig. 2. Temperature and salinity profiles of the water column during sampling (1300 h). (a) 24 Apr; (b) 25 Jun; (c) 28 Jul; (d) 22 Aug 1986

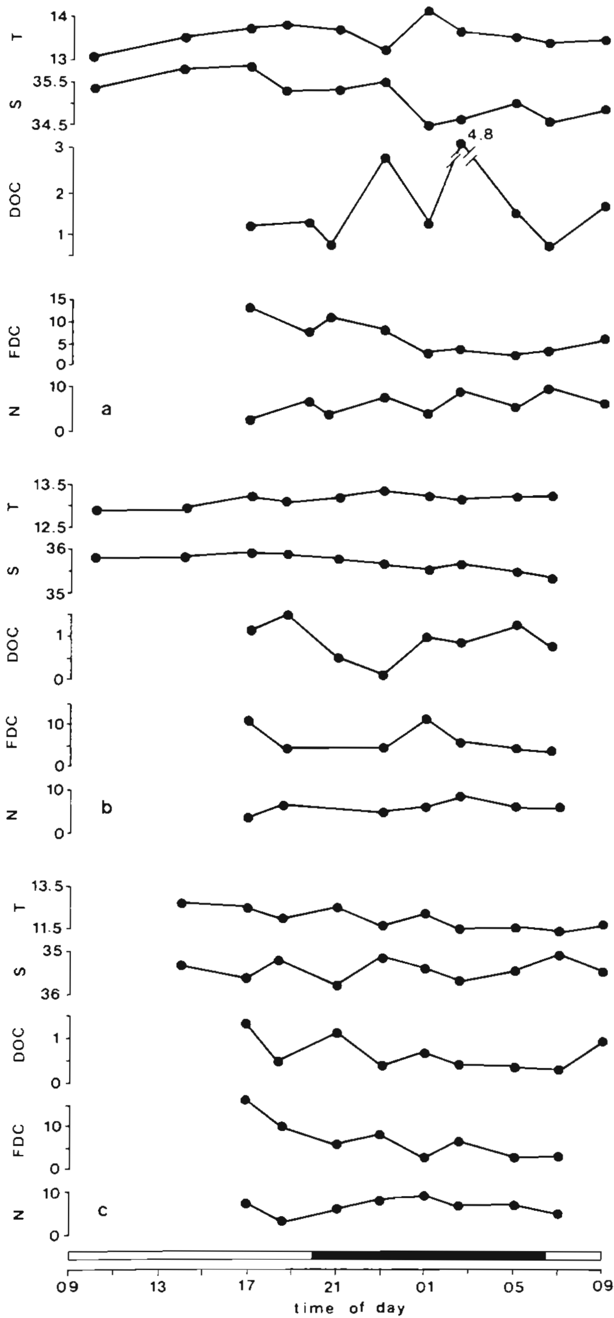
(Fig. 3) and June (Fig. 4) of several depth layers over the diel cycle (July data not shown; in August only DOC was measured in addition to the physical parameters).

Surface water layer

One of the general trends in the surface layer (0.5 m sampling depth) regardless of sampling date was the low DOC content of approx. 1 mg l^{-1} during daytime with an early morning peak in DOC of up to 5 mg l^{-1} around 0500 h. This morning peak was observed in 3 out of 4 diel cycles (Fig. 4a); only the April DOC cycle deviated from this pattern with generally high DOC contents of the surface waters during night-time (Fig. 3a). Bacterial abundance remained fairly constant over the diel cycles; however, bacterial activity as estimated by FDC showed pronounced diel cycles with peaks in late afternoon and evening. This pattern was consistent in all 3 days of measurement.

The 5 to 7 m water layer

This layer was well above the pycnocline during all diel measurements (Fig. 2). As in the surface layer, DOC showed a marked early morning peak with values up to 10 mg l^{-1} (Fig. 4b). Bacterial activity declined in the evening with lowest FDC around midnight. In April, however, about 12 % of the bacterial population was in a dividing stage at 0100 h. Corresponding to the



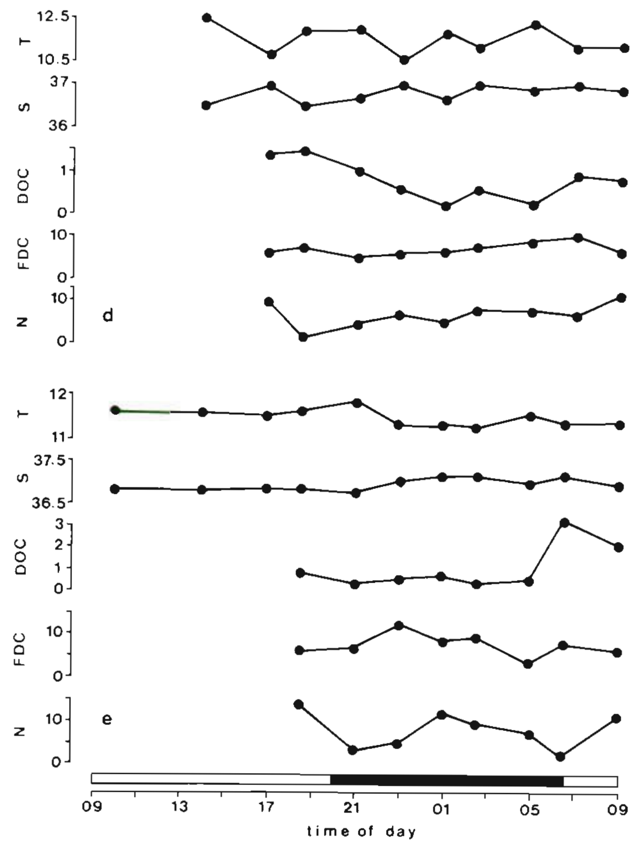


Fig. 3. Time courses of measured parameters on 24–25 Apr 1986 in (a) surface layer; (b) 5 m layer; (c) 15 m layer; (d) 16 m layer; (e) 17 m layer. Temperature (T) given in °C, salinity (S) in ‰, DOC in mg C l^{-1}, FDC in %, and bacterial numbers (N) in $n \times 10^5$ cells ml^{-1}. Dark horizontal bar indicates night-time

morning DOC peak, FDC values increased again at this time and varied only in a very narrow range over the entire diurnal cycle.

Pycnocline layer

DOC concentrations approached those of the overlying layers, with the same decreasing trends during night and peaks in the morning (Fig. 3c & 4c). FDC values remained high throughout the diel cycles, occasionally with even higher rates during the night (Fig. 3d, e & 4c). Bacterial density in the pycnocline layer exhibited larger fluctuations than in the overlying water body.

Dialysis bag incubations

In June, concurrently with the diel parameter measurements, bacterial secondary production was estimated by means of 2 μm filtered seawater filled in dialysis bags and incubated for 24 h. In 5 m depth,

bacterial density within the dialysis bags increased from 3.37×10^5 to 10.15×10^5 cells ml^{-1} (SD = 3.78, n = 4) or, expressed in turnover times of the bacterial population, a mean of 13.3 h (SD = 3.77, n = 4) is required for doubling bacterial density. While rods contributed 83 % to the initial bacterial abundance, this percentage declined to 67.5 % (SD = 6.4, n = 4) after the 24 h incubation period.

In the 10 m incubations bacterial abundance increased from 2.33×10^5 to 3.24×10^5 cells ml^{-1} (SD = 0.09, n = 4) which corresponds to a mean generation time of 42.5 h (SD = 3.8, n = 4). About 60 % of the initial bacterial population consisted of rod-shaped bacteria. This proportion remained more or less unchanged during the incubation: after 24 h, 59 % (SD = 9.8, n = 4) of the bacteria were identified as rods.

DISCUSSION

In the present study we sampled over 4 diel cycles from an anchored ship. It is unlikely that we sampled a strictly homogenous parcel of water over a diel cycle, although the temperature and salinity differences within the different layers over time are small. The possibility that an oscillating water body was sampled can be excluded since the main current direction roughly parallels the coast line, coming from the south and turning towards west in the Gulf of Trieste. We are aware of the fact that internal wave phenomena, in combination with patchiness, adds noise to our data. Although we sampled on a rather small scale in both space and time we probably lost some maximum and minimum concentrations of the parameters measured.

Nevertheless, it was possible to detect distinct diel variations in microbial parameters. DOC concentrations reached maximum values in the afternoon and at dawn regardless of depth. Only the surface layer in April deviated from this scheme and exhibited a more inconsistent pattern. The origin of the afternoon DOC maximum may be attributed to increased phytoplankton extracellular release during photosynthesis. Such an accumulation would imply that a greater amount of labile compounds of DOC are released by phytoplankton organisms diurnally than can be taken up by the microbial population. Higher concentrations and accumulations of various compounds of DOM during daytime have been recorded from other coastal areas, such as the Baltic Sea (Meyer-Reil et al. 1979, Hagström & Larsson 1984) as well as from the open ocean (Burney et al. 1982, Johnson et al. 1983, Burney 1986).

In most of the measurements the early morning peak exceeded the afternoon DOC maximum; especially in June, dawn DOC concentrations reached 10 mg l^{-1}. One explanation for this high DOC content in the morning could be the loss of phytoplankton cellular material during grazing by herbivores. Lampert & Taylor (1985) clearly demonstrated the influence of zooplankton diel migration on the distribution of nutrients in an eutrophic lake and Riemann et al. (1986) demonstrated that zooplankton grazing may increase the dissolved free amino acid pool potentially available for bacterial utilization. Early morning maxima of diel zooplankton feeding and excretion patterns were reported by Lorenzen (1967), Duval & Geen (1976), and Mackas & Bohrer (1976). In the layers above the pycnocline the observed diel pattern of DOC dynamics corresponded

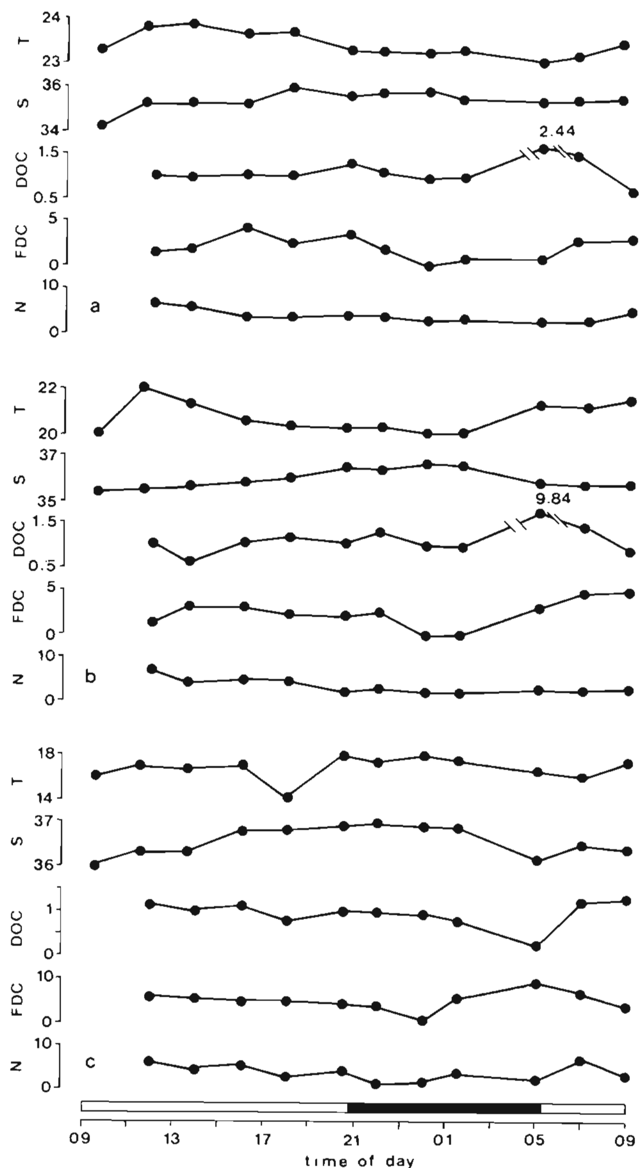


Fig. 4. Time courses of measured parameters on 25–26 Jun in (a) surface layer; (b) 5 m layer; (c) 15 m layer. For abbreviations see Fig. 3. Variables of the 2, 8 and 20 m layers are not shown

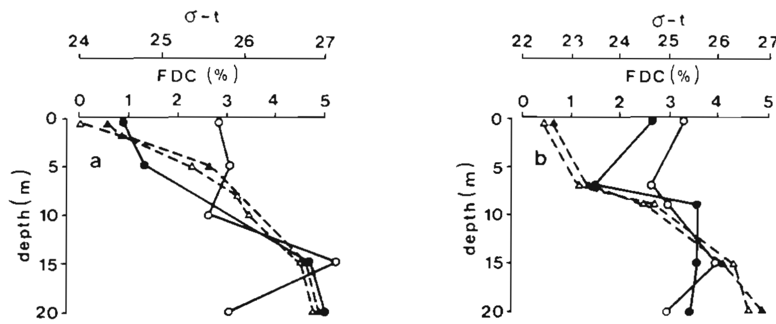


Fig. 5. Spatial and diel variation of FDC and sigma-t values in the stratified water column in (a) June and (b) July. Open symbols: daytime means; filled symbols: night-time; circles: FDC values in %; triangles: sigma-t. Each point represents the mean of 6 measurements during day and night

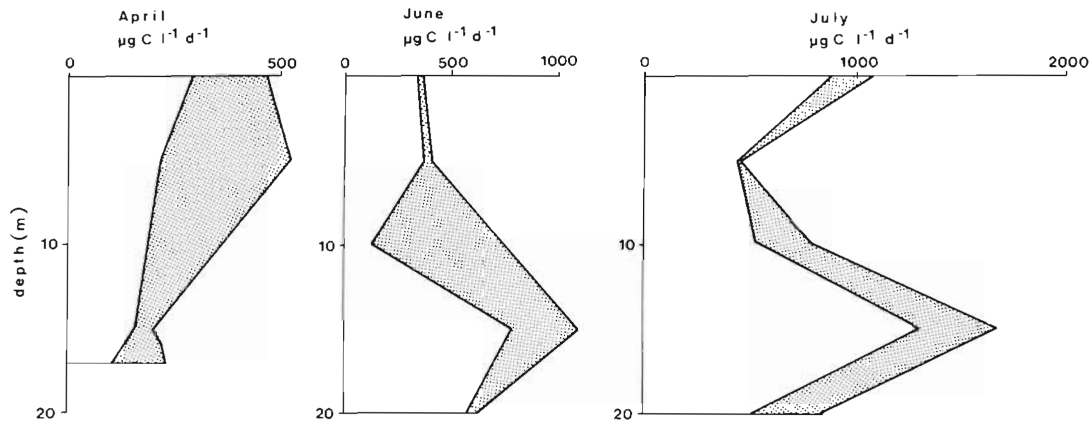


Fig. 6. Spatial variation in bacterial production in the water column during daytime (white area) and night (dotted area) calculated from FDC; daylength in April: 12 h, in June and July: 14.5 h

roughly to bacterial activity as measured by the FDC method, with higher FDC rates during daytime. Fig. 5 presents 2 examples (June & July) of pooled FDC day and night rates together with sigma-t values. A significant difference between day and night in FDC was obtained in all 3 diel cycles for the upper layers (down to 7 m) (Mann-Whitney, $p < 0.1$ in all cases). Converting these FDC values to bacterial turnover rates using the equations for different temperature regimes (Hagström & Larsson 1984), a mean generation time of 5.9 h was obtained in the surface layer during daytime (April), with a nocturnal value of 10.7 h. This difference was smallest in July, with a turnover rate for day and night of 5.3 and 6.7 h, respectively; in June the nocturnal generation time of 27.7 h was more than 4 times the diurnal (g = 6.1 h).

As indicated in Fig. 5 these pronounced diel differences in bacterial activity were largely restricted to the upper layers of the water column (see also Fig. 3 & 4), while in the vicinity of the pycnocline no such diel cycles in bacterial activity were detectable. In April the mean diurnal generation time in the pycnocline layer was 9.0 h (nocturnal: 12.3 h). These rates were therefore about one third higher than the corresponding surface layer values, although both during the day and night the FDC rates were higher at the pycnocline. Due to the different temperatures of the surface layer (13.5 °C) and pycnocline (11.8 °C), different equations (for 15 and 10 °C, respectively) were used for calculating the turnover rates. Thus, the generation time obtained in the surface layer may be arbitrarily underestimated and that in the pycnocline overestimated. In all the other diel cycles generation times of the pycnocline bacterial population ranged from 3.1 to 5.4 h with almost no differences between day and night.

Bacterial volume estimates reveal no significant diel fluctuations. All volume estimations for single days were therefore pooled. The mean biovolume of rods was 0.45 μm^3 (SD = 0.18, n = 425), and of coccoid forms 0.06 μm^3 (SD = 0.019, n = 205). These values enable the estimation of bacterial production based on the FDC method and assuming the carbon-to-biovolume ratio given in Bratbak (1985). For this purpose mean FDC values were calculated separately for day and night periods and used for further calculation. Fig. 6 presents the estimated diel bacterial production and distinguishes between day and night production in different layers. Diel and spatial variation of bacterial production as well as monthly differences are obvious. Average production generally increased from April to July probably caused by a combination of increasing temperature and the senescent stage of the phyto-

plankton bloom in June and July. While nocturnal bacterial production in April proceeded at a relatively high level (45 to 60 % of diel production) throughout the uppermost 5 m layer, it declined dramatically in June and July. Nocturnal production contributed less than 20 % to the diel production in the top 5 m water body.

In contrast to April, overall diel production in June and July in the pycnocline layer exceeded that of the surface waters (Fig. 6). During these 2 mo, nocturnal bacterial production contributed about 20 to 30 % of the diel production. Although there were almost no differences in generation times between day- and night-time in the pycnocline layer, bacterial production was significantly lower during night due to reduced bacterial biomass probably caused by increased grazing pressure. This pattern of production (Fig. 6) indicates a tight coupling between phytoplankton primary production and bacterial activity; this was also observed by Linley et al. (1983), Lancelot & Billen (1984) and Fuhrman et al. (1985). However, the enhanced microbial activity in the pycnocline layer (June and July) indicates that this coupling is not restricted to dissolved production of phytoplankton (which should occur predominantly in the upper layers where favorable light conditions provoke higher photosynthesis) but that particulate production enhances microbial activity as well: the sinking velocity of senescent phytoplankton cells may be reduced in this zone leading to an accumulation of moribund cells. As a consequence of prolonged residence time in combination with disruption or lysis of phytoplankton in the more dense water body DOC is released from the cells. This enhances bacterial activity. Lancelot & Billen (1984) arrive at a similar conclusion in a study of phyto- and bacterioplankton coupling in the North Sea. Mitchell et al. (1985) calculated that nutrient-enriched microzones around phytoplankton cells are large enough only in the thermocline layer to enhance bacterial growth. The role of the pycnocline in retarding the settling velocity of senescent phytoplankton and thus providing nutrients for bacterioplankton is further supported by the generally lower bacterial production in the 20 m layer (2 to 3 m above bottom) although the DOC measurements as well as the bacterial biomass distribution pattern give an inconsistent pattern only occasionally reflecting the higher bacterial production in the pycnocline layer. Clearly more information is required on the organic constituents of the DOM pool before a conclusive picture can be drawn; the determination of additional parameters (micro- and macrozooplankton density and activity) of these distinct layers is also necessary.

If we assume that bacterial production estimations based on the FDC method (Fig. 6) accurately reflect true conditions (see discussion below), grazing pressure on bacteria seems to be high. Recent studies on flagellate grazing indicate that these Protozoa are capable of controlling bacterial biomass (Fenchel 1982, Azam et al. 1983, Fuhrman & McManus 1984). The nocturnal decline in bacterial biomass observed in this study is probably a combined effect of reduced production and flagellate grazing.

Currently, considerable efforts are being made on the precise determination of bacterial production. The use of various methods measuring different cellular activities (Riemann et al. 1984) makes a comparison of results difficult. New promising techniques have recently been developed (Kirchman et al. 1986). The incubation method using dialysis bags, however, has not received adequate attention. Its advantages over simple bottle incubations include avoidance of nutrient depletion during the course of incubation (dialysis bags with a molecular weight cut off of 12000 daltons were used), no significant wall-growth of bacteria, and successful deployment over at least one full diel cycle (Turley & Lochte 1985, Herndl et al. in press). One serious shortcoming is the necessity of filtration procedures to exclude bacterivores and the poorly understood effect of confinement on bacterial activity (Ferguson et al. 1984). In this study we used 2 µm Nuclepore filters through which almost the entire bacterial population passed. During epifluorescence microscopy only few flagellates were occasionally detected in samples drawn from dialysis bags after *in situ* incubation for 24 h.

Fig. 7a compares growth yields obtained by the FDC method over a diel cycle and by incubation of dialysis bags. In the 5 m layer the growth yields derived from FDC are similar to the mean growth rate obtained by incubations, at least during daytime. In the 10 m layer, however, the former exceed the latter over almost the entire cycle. Only around midnight are FDC-based growth rates lower than the mean rate obtained from the incubation technique. No data are available for the following morning; the FDC production estimate for the 10 m layer given in Fig. 7b is thus most likely slightly underestimated if we assume an activity trend similar to that observed in the 5 m layer.

Bacterial biomass production estimates based on FDC for the 5 and 10 m layer are 3 and 14 times higher, respectively, than estimates based on dialysis bag incubations. Newell & Fallon (1982) and Fuhrman et al. (1985) found production rates in seawater measured with FDC to be 2 to 7 times higher than the rates obtained with the ^{3}H-thymidine method; dialysis bag incubations thus seem to give similar production rates to thymidine incorporation. Riemann & Søndergaard (1984) reported values up to 21 times higher with the FDC than with the thymidine method in lakes. Possible

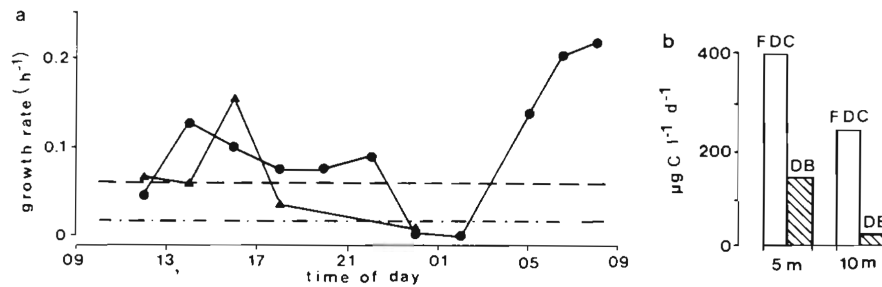


Fig. 7. Comparison between FDC and dialysis-bag-incubation based microbial parameters. (a) Variations in growth rates over the diel cycle obtained by FDC and average growth rate calculated from dialysis bag incubations. (•) FDC growth rates in the 5 m layer; (– – –) average growth rate from dialysis-bag-incubations. (▲) FDC growth rates in the 10 m layer; (–·–) corresponding mean of the dialysis bag-derived growth rate. (b) Difference in bacterial production estimates based on the FDC method and the dialysis bag (DB) technique for the 5 m and 10 m layers

reasons for these discrepancies are discussed in detail in Riemann et al. (1984). The preliminary results obtained in this study using dialysis bag incubations and the FDC method for bacterial production estimations are encouraging enough to justify a more thorough intercalibration of currently applied methods.

In summary, this study demonstrates the tight diel coupling between phytoplankton and bacterial activity. The observed variations of microbial parameters within different layers of the shallow water body examined point to the importance of small-scale investigations in both time and space. Although the water body was not uniform over time, the results obtained vary only in a narrow range, with similar trends even among different months. Similar diel investigations including more microbial variables and smaller depth-intervals are planned to elucidate the diel rhythm of bacterioplankton in different layers of stratified water columns.

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