DIEL CYCLES IN SINGLE CELL ACTIVITY OF PROKARYOTES IN THE WESTERN MEDITERRANEAN SEA

Eva Sintes * and Gerhard J. Herndl
Department of Biological Oceanography, Royal Netherlands Institute of Sea Research (NIOZ), The Netherlands - esintes@nioz.nl

Abstract
The abundance and activity parameters of prokaryotic communities were studied over four diel cycles in an anticyclonic eddy in the Western Mediterranean Sea. The prokaryotes from the different depths expressed diel variability in all the studied parameters. The prokaryotic community from the deep chlorophyll maximum (DCM) fluctuated in cell size, with larger cells appearing from the morning until noon, followed by the increase in the relative contribution and single-cell-activity of small cells. The percentage of prokaryotic cells taking up leucine showed no significant trend during the day and the single-cell uptake was higher in the early morning and around noon, however, distinct activity patterns were recorded for specific prokaryotic groups.
Keywords : Bacteria, Western Mediterranean, Open Sea.

Introduction
Diel cycles in prokaryotic abundance and production have been reported for several marine environments (e.g. [1]), with peaks in activity in the early morning or in the evening. These diel dynamics in prokaryotic heterotrophic activity are governed by phytoplankton production providing labile dissolved organic matter (DOM) to bacterioplankton and by ultraviolet radiation. While phytoplankton-derived DOM stimulates bacterioplankton activity, UV radiation inhibits it [2, 3].
The main objective of this study was to determine whether these diel cycles, if present, were expressed in the same way by the different prokaryotic groups at a single-cell level or whether there are interspecific differences in the activity pattern.

Material and Methods
An anticyclonic eddy located in the Western Mediterranean Sea was followed from 23 Sep to 6 Oct 2003 using a drifting buoy. Diel cycles of different prokaryotic parameters (abundance, production, actively respiring cells, abundance of cells with damaged membrane, MICRO-CARD-FISH) were determined by taking samples from six depths (near-surface: 5, 10, 20, 30 m), deep chlorophyll maximum (DCM, at about 50 m depth) and at the bottom of the euphotic layer at around 100 m depth approximately every 4 h over diel cycles. Using microautoradiography, the silver grain area associated with the prokaryotic cells taking up leucine has been used as an indicator of single cell activity [4, 5] at the DCM during one diel cycle.

Results and Discussion
The abundance and phylogenetic composition of some major groups of bacteria (as determined by fluorescence in situ hybridization, FISH) was fairly stable during the diel cycles, with lowest abundance at 100 m depth (mean 2.5 x 10^5 cells ml^{-1}) and highest abundance in the DCM with an average of 7.4 x 10^5 cells ml^{-1} and increasing abundance from 5 m depth to 30 m depths (Table 1). The percentage of cells with a high nucleic acid content followed a similar trend (Table 1).
Although the different prokaryotic parameters varied during the day, no clear prokaryotic diel cycles were found at any of the studied depths. The percentage of cells of the prokaryotic community of the DCM taking up leucine showed no significant differences during the 4th diel cycle, while the silver grain area of the DAPI-stained cells taking up leucine were highest in the early morning and at noon. Bacteria dominated the community (48 %) followed by Euryarchaea (17 %) and Crenarchaea (7 %). The SAR11 clade and Cytophaga-Flavobacter group represented 25 % and 16 %, respectively, of the prokaryotic community, while the Roseobacter cluster represented only 4 % and SAR86 was hardly detected. Single-cell activity of Bacteria showed no clear trend during the day, but members of the Cytophaga-Flavobacter cluster exhibited high cell activities during early morning and afternoon. Archaeal groups exhibited higher cell activities in the early morning and at noon (Crenarchaea) or afternoon (Euryarchaea). The prokaryotic community size structure showed three main peaks, at size classes of 0.12-0.14, 0.16-0.18 and 0.20-0.22 μm equivalent spherical diameter (ESD) and was quite stable from early morning until noon. From the early morning towards noon, some larger cells appeared, followed by an increase in the relative abundance of the 0.12-0.14 μm ESD size class during the afternoon, decreasing again towards the next morning. This pattern would be in accordance with an increase in cell size during the day and division during the afternoon. The dynamics of single-cell activity per size class was more variable. Higher single-cell activities were found at noon and early morning, and in the larger size classes. Excluding the larger sizes, an increase in single-cell activity was found from the smaller sizes towards the 0.26-0.28 μm ESD during the morning, afterwards the higher cell activities were found in smaller size classes coinciding with the increase in the abundance of these small size classes.
In summary, diel dynamics of the abundance and activity of the prokaryotic community were variable in the open Mediterranean Sea. The percentage of cells inhabiting the DCM and taking up leucine was stable during the day, but the single-cell activity was higher in the early morning and noon. Different prokaryotic groups showed different diel dynamics in single-cell activity.

Tab. 1. Average ($\pm$ SD) of prokaryotic abundance and activity over the different depths. * The % of cells taking up leucine corresponds to one time point (6:00 am).

	10^5 Cells ml^{-1}	% HNA	% CTC+	% Sytox	nmol Leu l^{-1} d^{-1}	amol Leu $cell^{-1}$ d^{-1}	% cells taking up Leucine*
100 m	2.5±0.9	43.8±2.2	6.9±7.9	12.7±5.7	0.15±0.08	0.66±0.41	40.16
DCM	7.4±1.6	51.0±3.7	8.7±7.0	12.6±5.4	1.47±0.50	2.12±0.94	47.40
30 m	6.1±1.4	48.8±3.3		8.3±3.1	0.83±0.23	1.44±0.51	
20 m	5.1±1.3	44.1±2.6		7.9±3.1	1.02±0.14	2.21±0.83	
10 m	4.7±1.3	43.8±3.0		8.0±2.6	0.85±0.17	1.98±0.73	
5 m	4.4±1.2	43.5±2.2	5.3±5.3	8.0±2.6	0.84±0.21	2.08±0.86	54.42

References

1 - Kuypers, B., G. J. Van Noort, J. Vosjan and G. J. Herndl. 2000. Diel periodicity of bacterioplankton in the euphotic zone of the subtropical Atlantic Ocean. *Mar. Ecol. Prog. Ser.* 201: 13-25.
2 - Kaiser E. and G.J. Herndl. 1997. Rapid recovery of marine bacterioplankton activity after inhibition by UV radiation in coastal waters. *Appl. Environ. Microbiol.* 63(10): 4026-4031.
3 - Obernosterer, I., B. Reitner and G.J. Herndl. 1999. Contrasting effects of solar radiation on dissolved organic matter and itsbioavailability to marine bacterioplankton. *Limnol. Oceanogr.* 44 (7):1645-1654.
4 - Cottrell, M. T. and Kirchman, D. L. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. *Limnol. Oceanogr.*, 48: 168-178.
5 - Sintes, E. and Herndl, G. J. 2006. Quantifying substrate uptake by individual cells of marine bacterioplankton by catalized reporter deposition fluorescence in situ hybridization combined with microautoradiography. *Appl. Environ. Microbiol.*, 72: 7022-7028.