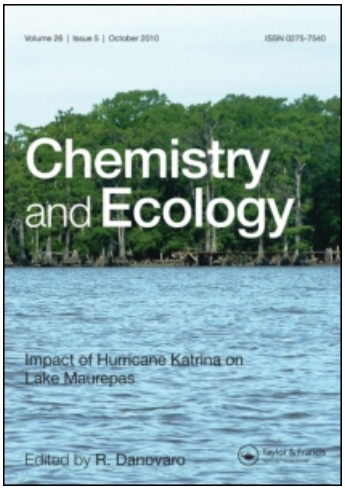

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DEEP-CHLOROPHYLL MAXIMUM TIME SERIES IN THE AUGUSTA GULF (IONIAN SEA): MICROBIAL COMMUNITY STRUCTURES AND FUNCTIONS

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An integrated study was carried out to follow the temporal evolution of microbiological parameters during a 48 h period, in relation to the deep chlorophyll maximum (DCM) at a coastal station. The micro-organisms showed an active role in the environment and a different distribution, without a clear diel cycle. The phytoplankton community, responsible for the DCM, consisted mainly of diatoms. Their distribution in relation to pycnocline showed an opposite trend with respect to picophytoplankton. Total bacterioplankton contributed to enzymatic degradation of particulated organic carbon (by producing β-glucosidase and aminopeptidase), with peaks related to changes in the main water current. We estimated that about 25% of particulate organic carbon per day may be hydrolysed by bacteria. The living bacterioplankton represented 20% of the total. The picophytoplankton fraction contributed significantly to the high values of alkaline phosphatase, suggesting a fast P regeneration. Respiration showed significant correlations with the physical and chemical parameters as well as with the different planktonic fractions.

Keywords: Bacteria; Phytoplankton; Microzooplankton; Enzymatic activities; Particulated matter; Respiration

1 INTRODUCTION

Phytoplankton and bacteria interactions play a crucial role in important processes such as carbon fluxes and nutrient regeneration. Organic matter, produced by the autotrophic component, moves via dissolved organic matter into bacteria and the microbial loop, while remineralization by bacteria supplies nutrients to phytoplankton (Azam, 1998). In the short timescale, the route by which fixed C becomes dissolved and available to bacteria is mainly due to phytoplankton exudation or sloppy feeding. This suggests that heterotrophic bacteria depend on the organic carbon supply produced by autotrophs with consequent adaptation of their metabolism. Nevertheless, this direct relationship is not always evident (Azam *et al.*, 1993). Since heterotrophic bacteria cannot directly use high-molecular-weight compounds, bacterial hydrolysis is recognized as a limiting step in organic-matter utilization (Chròst *et al.*, 1989; Hoppe *et al.*, 2002).

Moreover, micro-organisms react quickly to changes in the environment: phytoplankton diel cycles are related to the light cycle, while heterotrophic bacterioplankton react to

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variations in organic substrates availability. So, short-term variations in phytoplankton and bacterial activity can have a great influence in the C cycle, especially in coastal waters, where biological activities may be enhanced.

In oceanic environments, where substrate supply is low, the coupling between producers and consumers in the diel cycle is evident during the hours of daylight (Fuhrman *et al.*, 1985), even though bacteria and phytoplankton may compete when nutrients become limited (Azam, 1998; Kuipers *et al.*, 2000; Sala *et al.*, 2002).

In coastal areas, less coupling between producers and consumers was reported because other sources of organic carbon come from land inputs, and bacteria may utilize allochthonous carbon independently of primary production (Gasol *et al.*, 1998).

All the above processes, such as the release of dissolved organic matter by algae exudation and lysis, grazing and sloppy feeding by zooplankton, etc., occur mainly at the deep chlorophyll maximum (DCM) layer, characterized by a significant phytoplankton growth due to the simultaneous availability of nutrients and light (Cho and Azam, 1990).

During a multidisciplinary study in a coastal semi-enclosed gulf of the Ionian Sea (the Augusta bay), the microbiological parameters were investigated on a short timescale to determine whether variation of bacteria depends on variation of phytoplankton or whether they respond to a variation of allochthonous carbon supply.

The abundance of autotrophic (pico-, nano- and microphytoplankton) and heterotrophic components (total and living fractions of bacterioplankton, culturable bacteria, microzooplankton), together with particulate organic carbon and nitrogen (POC and PON) contents, were determined. Microbial activities (leucine aminopeptidase, β-glucosidase, alkaline phosphatase and respiration) were also measured.

The specific objectives of our study were to elucidate whether significant daily variations in the microbial populations occurred and how micro-organisms adapt their enzyme patterns of degradation and respiration to changes in environment.

2 MATERIALS AND METHODS

2.1 Sampling and Parameters Analysed

From 9 to 11 October 2001, a sampling cruise was carried out by the R/V *Tethis* in a coastal station located in the bay of Augusta, near the Sicilian coast of the Ionian Sea. The Aretusa station (37° 12.17′; 15° 14.54′) was chosen for a time series of observations. The experiment comprised 14 samplings performed during a 48 h period at approximately regular intervals and at four different depths (surface, above DCM, within DCM, below DCM) in the 20–60 m layer. Samples were collected using Seabird CTD, equipped with a Rosette sampler, with twelve 8 l Niskin bottles. The *in vivo* fluorescence peak increased from 25 to 40 m depth, so the DCM was affected by vertical changes in the peak during the experiment.

Chlorophyll (Chl-*a*) and nutrients (NO_3 and PO_4) estimations are described in Decembrini *et al.* (2004).

Phytoplankton was collected only on the first sampling day. Cell counts were obtained from 50 to 100 ml subsamples, preserved with Lugol's iodine solution, settled for 24–48 h, and examined under an inverted microscope (Labovert FS Leitz), using the method described by Utermöhl (1958). Algae, which were not identified either for the species or the genus, were assigned to suprageneric groups such as diatoms, coccolithophorids, dinoflagellates and phytoflagellates. For species identification, the principal taxonomic texts were those indicated in Zingone *et al.* (1990). As regards the phytoflagellates, a distinction

between photosynthetic and non-photosynthetic species was made using the information available in the literature (Chrétiennot-Dinet, 1990; Larsen and Sournia, 1991).

Seawater samples for bacterioplankton direct counts were fixed on board with formaldehyde (final concentration 2%) and stored in the dark at 4 °C.

The picophytoplankton direct count was done according to El Hag and Fogg (1986) using epifluorescence microscopy.

The bacterioplankton total count (TC) was carried out using DAPI according to Porter and Feig (1980). Bacteria with active cellular synthesis were determined by the direct viable count (DVC) procedure (Joux and LeBaron, 1997). The nucleoid-containing cell counts (NuCC) were performed according to Karner and Fuhrman (1997). Bacteria with intact membranes (L/D) were enumerated after staining with a Live/Dead BacLight Viability Kit (Molecular Probes, Inc.). The cells were enumerated at a magnification of 1000× under immersion oil by epifluorescent microscopy (Axioplan 2 Zeiss) equipped with a 100 W Hg lamp and DAPI filter set (G 365; FT 395; LP 420) for TC, DVC and NuCC procedures. For the L/D stain procedure, the live (green fluorescent) and dead (red fluorescent) cells were viewed separately with fluorescein (BP 450–490; FT 510; LP 520) and rhodamine (BP 546/12; FT 580; LP 590) filter sets. Cell counts were performed on a minimum of 20 randomly selected fields, and the concentrations were reported as mean value of cells ml^{-1}. Only two samplings (14.20 and 18.55 on the first day) were carried out to determine the living/active fraction.

For microzooplankton determination, 5 l samples from each depth were concentrated to 250 ml by slowly and gently pouring the water through a 10 μm mesh filter. Concentrated water samples were fixed with Lugol's solution (2% final concentration). Aliquots (100 ml) were settled in sedimentation chambers and examined with an inverted Zeiss microscope at 200× magnification. *Tintinnida* species were identified on the basis of lorica morphology following the descriptions of Rampi and Zattera (1982), while metazoan, protozoa non-ciliates and naked ciliates were counted following Fonda Umani and Milani (1990).

Extracellular enzyme activities (EEA) and in particular the activity rates of leucine aminopeptidase (LAP), β-glucosidase (β-GLU) and alkaline phosphatase (AP) were determined according to Hoppe *et al.* (1988). Triplicate 10 ml sub-samples were incubated for 3 h at *in situ* temperature with increasing concentrations (from 0.1 to 100 μM) of specific fluorogenic substrates (L-leucine-7-amido 4-methyl coumarin, leu-MCA, for LAP, 4-methylumbelliferyl-b-d-glucopyranoside, MUF-glu, for β-GLU, and 4-methylumbelliferyl-phosphate, MUF-phos, Sigma, for AP) to determine the maximum velocity (V_{max}) of hydrolysis. The increase in fluorescence was measured with a Hitachi F-2000 spectrofluorimeter at 380 nm (excitation) and 440 nm (emission) wavelengths, for LAP, and 365 nm (excitation) and 445 nm (emission), for β-GLU and AP. Calibration was performed with appropriate amounts of the standards MCA and MUF, respectively.

To estimate particulate organic matter (POC and PON), suitable quantities of sea water (500–2000 ml) were concentrated on Whatman GF/C filters. The filters were exposed to hydrochloric acid fumes for 24 h at room temperature and processed at 970 °C in a Perkin-Elmer CHN-Autoanalyzer 2400, using acetanilide as a standard (Iseki *et al.*, 1987).

Water samples for respiration rate determination were prefiltered through a 250 μm mesh net to remove large particles and were concentrated on GF/F Whatman glass fibre filters. The filters were immediately stored in liquid nitrogen until analysis (<45 d) to prevent enzymatic decay (Ahmed *et al.*, 1976). Respiration rates and metabolic production of CO_2 were obtained by electron transport systems assay (ETS) performed according to the tetrazolium reduction technique extensively described by Packard (1971) and modified by Kenner and Ahmed (1975) for the microplankton community.

Culturable heterotrophic bacteria density, expressed as CFU, was estimated by spreading 0.1 ml sample on Marine Agar plates (MA) and incubating at 20 ± 1 °C for 7 ± 1 d.

2.2 Conversion Factors

We used a 20 fg C cell^{-1} factor to convert the bacterioplankton direct count into bacterial biomass (BB) according to Lee and Fuhrman (1987). A conversion factor of 72 was used to convert amino acids and glucose released in carbon according to Hoppe *et al.* (1988); in a similar way, the potential phosphatase activity was converted into potential phosphorus released by a factor of 31.

Phytoplankton carbon biomass (PhB) was calculated from chlorophyll *a* concentration using the C:Chl ratio of 50, which is a reasonable approximation of the range 20–125 from fieldwork. Data were statistically analysed by the coefficient of variation (C.V.) and by the Pearson correlation coefficient. To check the existence of daily cycles, the data from light and dark periods of two consecutive days were tested by ANOVA.

3 RESULTS

During the sampling days, different patterns were observed in the measured parameters. In Table I, the mean values and C.V. of the microbiological and biochemical parameters are reported.

The importance of circulation in relation to time variability at the station Aretusa (37° 12.17′; 15° 14.54′) is described in the paper of Raffa and Hopkins (2004). In brief, they considered four phases: at the beginning of Phase I (from 11.00, 9 Oct. to 01.00, 10 Oct.) a maximum current velocity towards south occurred in the depth range of the DCM, and this was coincident with the most prominent nitrate enrichment. Another shorter southern flow event occurred at the beginning of Phase IV (01.00, 11 Oct. until 09.00, 11 Oct.) and was associated with a weaker enrichment of nitrate. During Phase III, the flow in the DCM was northward with a decrease in nitrate concentration.

Temperature, ranging from 16.20 to 23.83 °C, showed narrow variations in the depth profile (C.V. = 11.3%). A particular feature of Aretusa station was the absence of any terrestrial influence. The salinity (ranging from 38.45 to 38.62) and density variations are described by Raffa and Hopkins (2004).

3.1 Phytoplankton Density and Composition

The phytoplankton densities showed a mean value of $8.15 \pm 0.67 \times 10^4$ cells l^{-1}, ranging between 2.45 (at 22.35, 73 m) and 16.1×10^4 cells l^{-1} (at 22.35, 52 m). The phytoplankton community consisted mainly of diatoms (51.3%) and phytoflagellates (nanophytoplankton, 45.7%). Dinoflagellates and coccolithophorids represented 2.1% and 0.9%, respectively. Within the 20–50 m layer, diatoms were dominant and reached 81.4% of the phytoplankton community (at 22.35, 40 m). Phytoflagellates, mainly represented by small (<10 μm) forms of uncertain taxonomic classification, showed a wide distribution within the entire water column. A total of 69 phytoplankton species were identified, of which 46 were diatoms, 19 dinoflagellates, two coccolithophorids and two phytoflagellates. The most representative species of the community belonged to diatoms, characterized by a high biodiversity and mainly represented by *Chaetoceros* spp., *Leptocylindrus danicus* and *Pseudo-nitzschia pseudodelicatissima* (Tab. II).

TABLE I Mean values and coefficients of variation (C.V.) of microbiological and biochemical parameters in the water column, during the two sampling days (the average C.V. along the water column and over time are also shown).

	9 October							
Sampling n	*35*	*38*	*41*	*42*	*45*	*46*	*47*	*50*
hour	*11.25*	*14.20*	*16.15*	*18.55*	*22.35*	*04.15*	*07.15*	*09.30*
Total picoplankton (TC)								
cell ml^{-1}	3.3E+05	4.5E+05	2.0E+05	2.6E+05	1.9E+05	2.8E+05	2.8E+05	1.9E+05
C.V.%	36.5	65.3	37.8	50.6	24.7	26.9	27.7	16.8
Picophytoplankton (PPDC)								
cell ml^{-1}	3.6E+04	2.8E+04	2.6E+04	1.7E+04	2.4E+04	3.5E+04	3.1E+04	2.0E+04
C.V.%	67.0	79.8	78.9	65.7	86.7	58.8	78.3	105.0
Culturable bacteria (MA)								
CFU ml^{-1}	420	289	128	158	105	116	94	93
C.V.%	135	56	49	106	14	24	41	47
Total phytoplankton								
cell l^{-1}	1.E+05	8.E+04	6.E + 04	7.E+04	1.E+05	7.E+04	5.E+04	
C.V.%	13	20	56	59	35	17	52	
Total microzooplankton								
cell l^{-1}			27.0	25.3	49.8		44.0	62.5
C.V.%			38.4	43.3	52.3		31.0	34.8
β-Glucosidase (β-GLU)								
nM l^{-1} h^{-1}	1.0	108.2	1.5	5.6	2.7	3.8	3.2	1.8
C.V.%	66.7	197.2	78.0	163.7	13.7	34.1	58.9	109.6
Aminopeptidase (LAP)								
nM l^{-1} h^{-1}	174.46	784.94		143.05	21.26	124.13	120.29	56.18
C.V.%	11.25	105.63		43.37	64.90	35.82	50.58	20.47
Phosphatase (AP)								
nM l^{-1} h^{-1}	576.44	591.25	566.12	728.99	1052.54	1110.81	537.30	329.08
C.V.%	63.04	75.97	70.82	75.49	80.89	45.55	73.93	118.81
Respiration (ETS)								
μl O_2 h^{-1} l^{-1}	0.77	0.45	0.40	0.30	0.47	0.46	0.50	0.44
C.V.%	53.38	36.05	33.28	60.40	28.52	36.14	18.89	40.17
POC								
μg l^{-1}	81.7	88.4	64.2	85.2	85.0	75.1	57.1	50.3
C.V.%	30.2	22.6	25.2	31.7	10.6	18.8	31.4	19.6
PON								
μg l^{-1}	10.6	15.9	7.5	12.7	13.9	6.6	15.3	6.4
C.V.%	45.7	36.4	8.5	21.8	24.5	56.2	15.7	39.1
C/N								
	8.5	5.8	8.7	7.2	6.6	14.2	3.8	8.5
C.V.%	32.3	17.1	33.4	48.3	39.2	51.8	37.4	29.5

(*continued*)

TABLE I Continued.

Sampling n	*53*	*56*	*57*	*60*	*11 October 61*	*64*	*Mean C.V.% with depth*	*Mean C.V.% over time*
hour	*14.05*	*16.44*	*19.42*	*22.01*	*07.05*	*09.24*		
Total picoplankton (TC)								
cell ml^{-1}	1.9E+05	2.2E+05	2.4E+05	2.3E+05	1.9E+05	1.8E+05		
C.V.%	18.6	20.6	29.7	35.4	49.4	30.5	33.6	43.0
Picophytoplankton (PPDC)								
cell ml^{-1}	1.6E+04	2.8E04	2.9E+04	2.6E+04	3.2E+04	3.2E+04		
C.V.%	79.3	105.8	110.5	87.7	68.1	87.0	82.8	52.6
Culturable bacteria (MA)								
CFU ml^{-1}	345	304	325	250	9	51		
C.V.%	109	144	45	52	44	155	72.9	107.0
Total phytoplankton								
cell l^{-1}								
C.V.%							36.1	38.9
Total microzooplankton								
cell l^{-1}	63.8	52.5	51.5	54.0	69.5	60.5		
C.V.%	50.3	35.9	35.5	34.3	16.7	28.20	36.4	39.3
β-Glucosidase (β-GLU)								
nM l^{-1} h^{-1}	6.1	3.0	1.0	2.2	12.2	0.5		
C.V.%	185.1	71.8	76.3	73.5	69.5	124.1	94.4	207.5
Aminopeptidase (LAP)								
nM l^{-1} h^{-1}	269.49	115.29	184.77	188.62	420.67	129.43		
C.V.%	90.85	42.21	59.68	30.09	38.35	78.51	51.7	110.5
Phosphatase (AP)								
nM l^{-1} h^{-1}	838.93	383.70	486.39	546.25	1230.41	630.53		
C.V.%	85.23	104.33	63.46	92.77	74.80	55.45	77.2	52.1
Respiration (ETS)								
μl O_2 h^{-1} l^{-1}	0.52	0.64	0.65	0.66	0.60	0.44		
C.V.%	22.48	33.56	15.23	31.14	6.84	28.01	31.7	37.5
POC								
μg l^{-1}	61.5	70.1	70.7	44.7	39.0	49.8		
C.V.%	25.0	23.5	24.2	23.7	21.9	26.9	24.0	29.9
PON								
μg l^{-1}	8.2	6.0	10.4	4.9	8.0	14.6		
C.V.%	39.0	58.8	32.5	52.6	22.5	11.1	35.1	45.0
C/N								
	8.2	15.4	7.1	11.2	5.0	3.4		
C.V.%	37.0	66.8	26.4	47.9	27.5	23.1	37.0	56.5

TABLE II List of the most representative phytoplanktonic taxa in the Augusta Gulf.

	Avg (*cell* × 10^3 l^{-1})	*S.D.* (*cell* × 10^3 l^{-1})	*Max* (*cells* × 10^3 l^{-1})	*%*	*n*
Diatoms					
Bacteriastrum delicatulum	3.26	0.34	7.30	1.92	13
Chaetoceros affinis	2.53	0.18	4.38	1.61	14
Chaetoceros curvisetus	6.94	0.86	20.44	6.93	22
Chaetoceros decipiens	4.18	0.42	9.49	3.42	18
Chaetoceros didymus	4.80	0.65	10.95	1.53	7
Chaetoceros spp.	6.84	1.11	24.46	8.39	27
Cylindrotheca closterium	1.51	0.20	4.38	1.58	23
Guinardia striata	2.27	0.25	5.84	2.79	27
Leptocylindrus danicus	2.95	0.37	7.30	3.48	26
Leptocylindrus minimus	1.95	0.27	4.38	1.06	12
Proboscia alata	0.98	0.08	1.46	1.03	23
Pseudo-nitzschia fraudolenta	1.85	0.28	5.84	1.34	16
Pseudo-nitzschia pseudodelicatissima	2.92	0.35	7.30	3.18	24
Thalassionema bacillare	2.51	0.46	10.22	2.97	26
Dinoflagellates					
Gymnodinium spp.	1.03	0.12	2.19	0.51	11
Scrippsiella trochoidea	0.15	0.07	1.46	0.18	27
Coccolithophorids					
Emiliania huxleyi	0.94	0.07	1.46	0.60	14
Phytoflagellates					
Cryptophyceans	2.29	0.30	4.38	0.73	7
Undetermined phytoflagellates <10 μm	38.49	4.93	96.36	38.48	22
Undetermined phytoflagellates >10 μm	14.11	2.83	45.26	7.70	12

Note: Avg: averaged cell density; S.D.: standard deviation; Max: maximum density; %: percentage of present organisms; *n*: frequency.

Diatoms seemed to be responsible for the DCM; in fact, during the first sampling day, we observed a significant correlation between diatoms and fluorescence ($r = 0.50$, $n = 21$, $P < 0.05$). Nutrients did not show a direct correlation with phytoplankton dynamics, with the exception of ammonia concentration, which was correlated to coccolithophorids ($r = 0.66$, $n = 18$, $P < 0.05$). The correlation between nanophytoplankton and picophytoplankton was significant ($r = 0.49$, $n = 19$, $P < 0.05$). Furthermore, total phytoplankton and diatoms were negatively correlated with the Protozoa not Ciliophora ($r = -0.81$ and $r = -0.71$, respectively, $n = 12$, $P < 0.01$).

3.2 Picophytoplankton Abundance

Picophytoplankton abundance varied from 0.39×10^4 (at 19.42, 45 m, second sampling day) to 7.29×10^4 cells ml^{-1} (at 19.42, 20 m, first sampling day); the cell densities decreased with the depth of the water column, from 5.21 to 1.02×10^4 cells ml^{-1}. The picophytoplankton densities showed a high C.V. with time and depth of the water column (C.V. = 52.6 and 82.8%, respectively). Nevertheless, the ANOVA did not show any significant difference between light and dark periods. The picophytoplankton abundance, as a percentage of the total bacterioplankton, increased with time, representing about 6–18%, with no clear link to nictemeral cycles. It was significantly related to POC ($r = 0.28$, $n = 53$, $P < 0.05$), Ciliofora and Tintinnida ($r = 0.39$, $r = 0.43$, respectively, $n = 56$, $P < 0.05$); moreover, it was involved in the microbial processes of phosphatase production and respiration ($r = 0.41$ and $r = 0.40$, respectively, $n = 53$, $P < 0.01$).

Diatoms and picophytoplankton had opposite trends in relation to the pycnocline (Fig. 1). As a matter of fact, picophytoplankton showed a negative correlation with density and prevailed in the upper layer (density <27.60). On the contrary, diatoms prevailed in the layer between densities of 27.60 and 28.2, mainly in correspondence with the fluorescence peak.

3.3 Microzooplankton Abundance

Total microzooplankton abundance showed slight variations along both water column and sampling times (C.V. = 36.4 and 39.3%, respectively); the maximum value (104 cells l^{-1}) was observed at 09.30 and 14.05 on the second day, at the surface and 52 m, respectively. Minimum values occurred on the first day at 16.15 and 18.55. The group distribution of microzooplankton showed a prevalence of the tintinnid, which reached a percentage of 68% without a clear diel cycle (Fig. 2); the other groups were: larvae of Metazoa that were abundant in every sampling (up to 57%) and Ciliophora not Tintinnida (up to 75% at 18.55, 35 m depth). In this study, the naked ciliates were probably underestimated, since the cells may have been damaged by the filtration method and lost through the net (Fonda Umani *et al.*, 1995). Tintinnida abundances were positively correlated with respiration, LAP and picophytoplankton ($r = 0.50$, $r = 0.45$, $r = 0.43$, respectively, $n = 44$, $P < 0.01$), but not with Chl-*a*. Protozoa, and were negatively correlated with diatoms and other phytoplankton fractions (see above), suggesting a possible control on phytoplankton abundance.

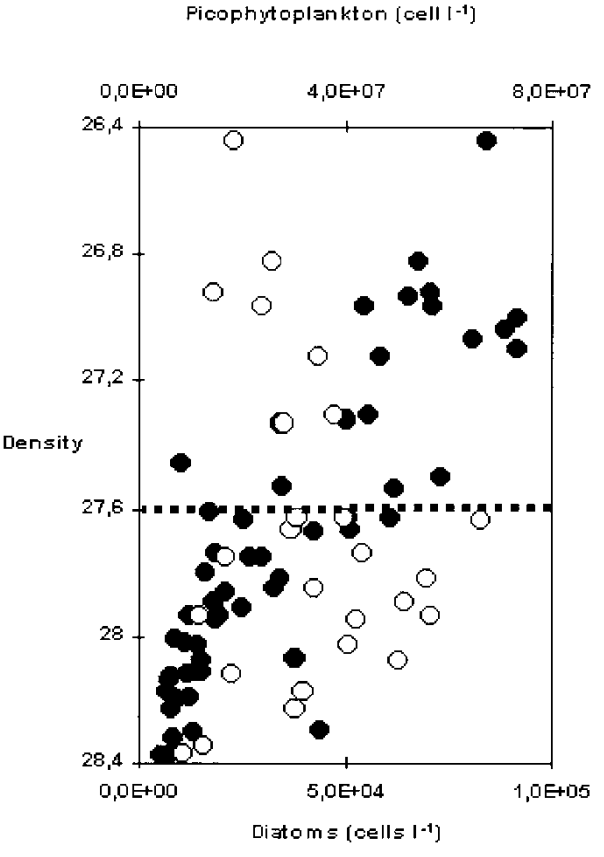

FIGURE 1 Diatoms (●) and picophytoplankton (○) distribution in relation to density.

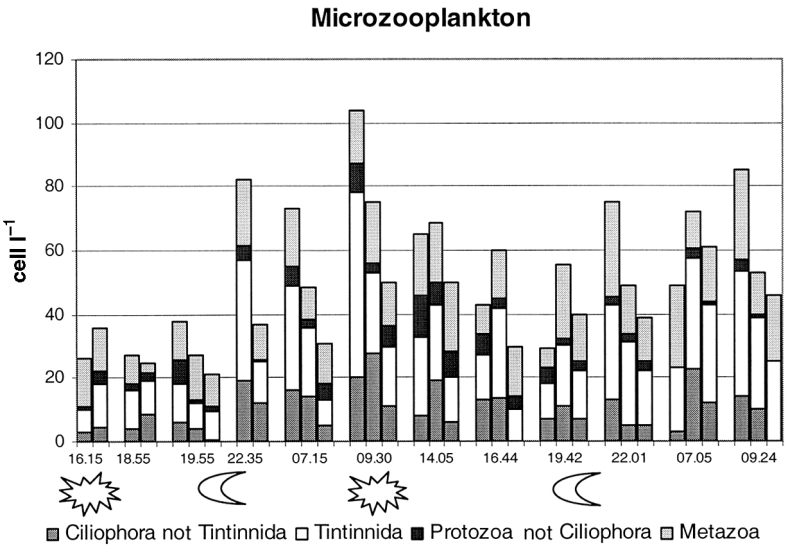

FIGURE 2 Microzooplankton density at different sampling times analysed in three depth layers: 1st bar above 24 m, 2nd bar between 25 and 55 m, 3rd bar below 56 m.

3.4 Bacterioplankton Distribution

Bacterial abundance (TC) showed low values, typical of oligotrophic oceanic regions. TC varied between 0.73×10^5 (at 7.05, 40 m, second sampling day) and 8.52×10^5 cells ml^{-1} (at 14.20, 20 m, first sampling day), without important variations in relation to depth, while a higher variability with time occurred (C.V. = 43.0). A significant variation between the two light periods of the consecutive days ($F = 8.18$, $n = 30$, $P < 0.05$) was observed, independently from DCM.

The values of the living fraction (NuCC and L/D) were similar in order of magnitude and in mean value. With respect to TC, they showed similar percentages (19.4% and 18.2%, respectively) and no significant difference, as deduced by ANOVA. The active cells (DVC) were 1% of TC, demonstrating that only a small fraction of total bacterioplankton is living and/or active at any time. The NuCC and L/D were two orders of magnitude greater than the culturable fraction, and the DVC exceeded CFU by one order of magnitude (Fig. 3). The correlations observed between these living fractions, nutrients and particulate matter confirmed their role in active processes (L/D vs. NO_3 $r = -0.75$, $n = 0.7$, $P < 0.05$; L/D vs. PON $r = -0.81$, $n = 7$, $P < 0.05$ and DVC vs. POC $r = 0.81$, $n = 7$, $P < 0.05$).

3.5 Enzyme Patterns

LAP mean values calculated for the whole water column ranged from 21.26 (at 22.35, on the first day) to 784.94 nM h^{-1} (at 14.20, on the first day); β-GLU mean values were between 0.489 (at 9.24, on the second day) and 108.17 nM h^{-1} (at 14.20, on the first day; Tab. I). In this latter sampling, peaks of both LAP (1955.1 nM h^{-1}) and β-GLU (428.12 nM h^{-1}) were observed in the upper layer (25 m), coinciding with a peak of total bacterioplankton density (8.52×10^5 cells l^{-1}) (Figs. 4A and B).

During the first day, the peaks of LAP activity were opposite to those of nitrates, which reached sufficient concentrations to sustain DCM (Decembrini *et al.*, 2004). In the afternoon

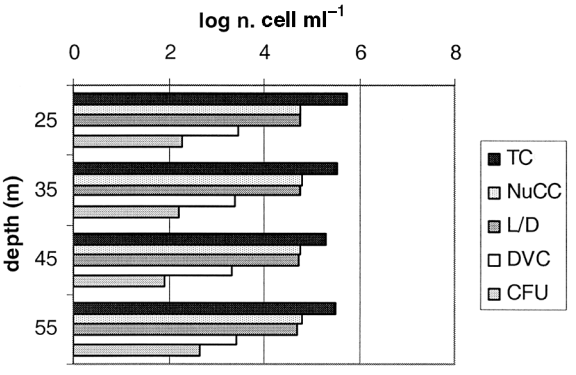

FIGURE 3 Comparison among bacterial density determined by different methods: total bacterial count by DAPI stain (TC); nucleoid containing cell count (NuCC); live bacterial count by L/D stain (L/D); direct viable count (DVC); culturable bacteria as CFU.

of the first sampling day (14.05), clear correspondences were observed between the peaks measured in chlorophyll biomass (Chl-*a*, 0.313 $\mu g\, l^{-1}$), POC, the enzymatic activities (LAP, β-GLU), total (TC) and culturable bacterial density (MA), suggesting a good balance between production and utilization processes.

On the second day, when the hydrological conditions changed, the nitrate concentrations showed an abrupt decrease (about 57%), while the LAP activity showed an increasing trend, with peaks at 14.15 and at 7.05. Even if the correlation was not significant, this opposite trend suggested that the low nitrate concentration could stimulate hydrolytic activity of proteins. This hypothesis was supported by the distribution of PON content (Fig. 4A).

No diel cycle, with clear differences between day and night, was observed in the extracellular enzyme activity. Only LAP activity showed a significant increase in the second day during the dark period (ANOVA $F = 7.52$, $n = 23$, $P < 0.01$). Moreover, enzymatic activities (β-GLU and LAP) were related to total bacterioplankton ($r = 0.67$ and $r = 0.68$, $n = 56$, $P < 0.01$, respectively), indicating that the metabolic activities of degradation were associated with bacterial biomass (Fig. 4A and B). The β-GLU activity was also well correlated with LAP ($r = 0.86$, $n = 56$, $P < 0.01$) and only during the first day to POC ($r = 0.44$, $n = 32$, $P < 0.01$). LAP activity showed a significant correlation with microzooplankton, in particular with Ciliophora and Tintinnida fractions ($r = 0.42$ and $r = 0.45$, $n = 54$, $P < 0.01$, respectively).

Alkaline phosphatase mean values, ranging from 329 to 1111 $nM\, h^{-1}$, were generally higher than LAP activity. The low peak of inorganic phosphate was observed in coincidence with peak of alkaline phosphatase activity, but the relationship was not significant (Fig. 4C). AP appeared to be significantly related, particularly on the first day, to trophic inputs, as suggested by the positive correlation with chlorophyll *a* and POC contents ($r = 0.53$ and $r = 0.51$, $n = 32$, $P < 0.01$, respectively). Moreover, the relationship with picophytoplankton suggested that there was a contribution of small autotrophs to phosphatase production (Fig. 5).

3.6 POC–PON Distribution

POC vertical concentration was fairly homogeneous during the observation period (Tab. I). The values ranged from 29.10 to 122.40 $\mu g\, l^{-1}$ at 18.55 in the upper layer. The daily evolution showed slight variations, with peaks in the afternoon on the first and second day (Fig. 4).

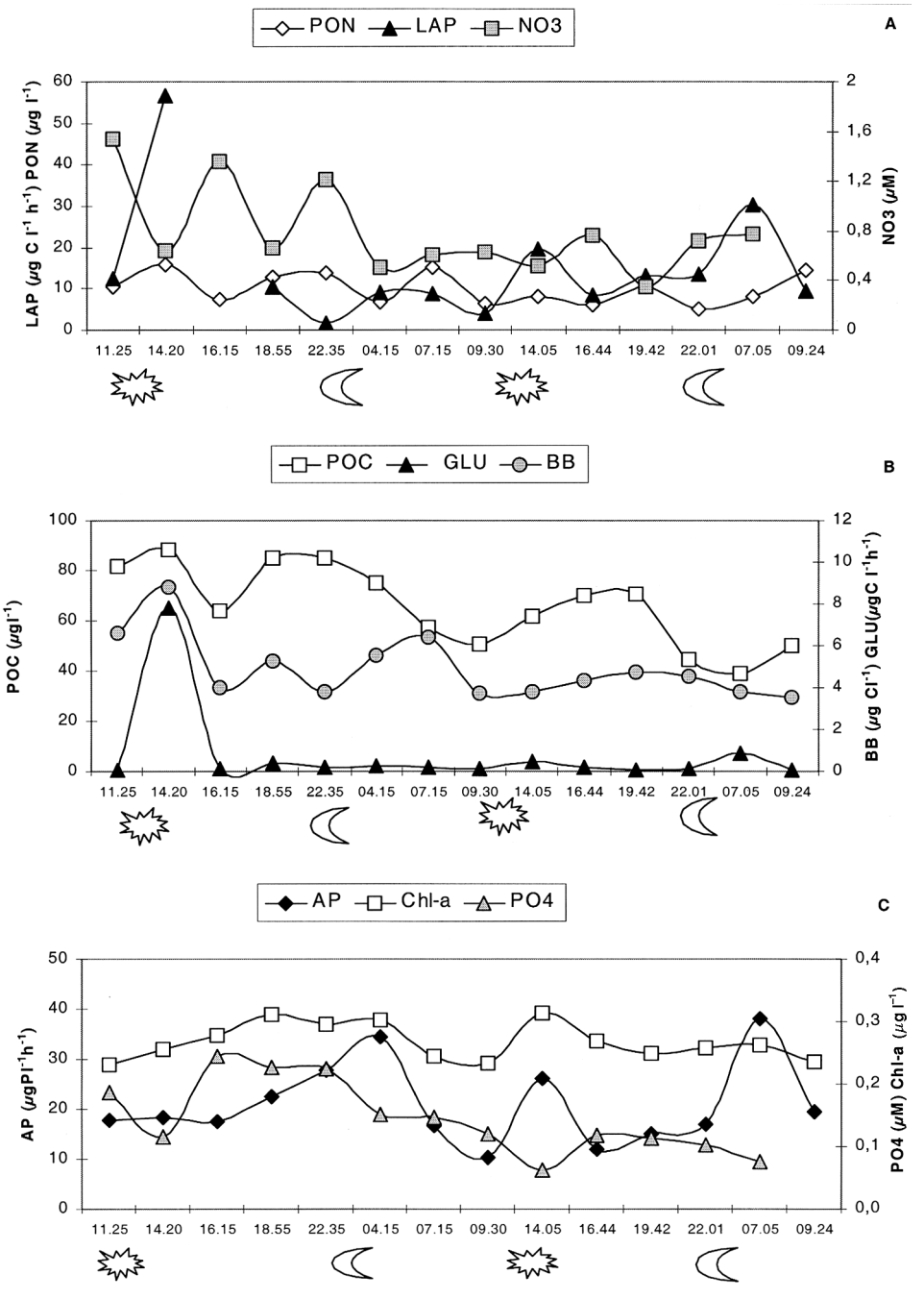

FIGURE 4 Daily behaviour of microbiological, chemical and biochemical parameters. (A) Leucine aminopeptidase (LAP) in relation to particulate nitrogen (PON) and nitrate contents (NO_3). (B) β-Glucosidase (GLU) in relation to particulate organic carbon (POC) content and bacterial biomass (BB). (C) Alkaline phosphatase (AP) in relation to inorganic phosphorus (PO_4) and chlorophyll-*a* (Chl-*a*) contents.

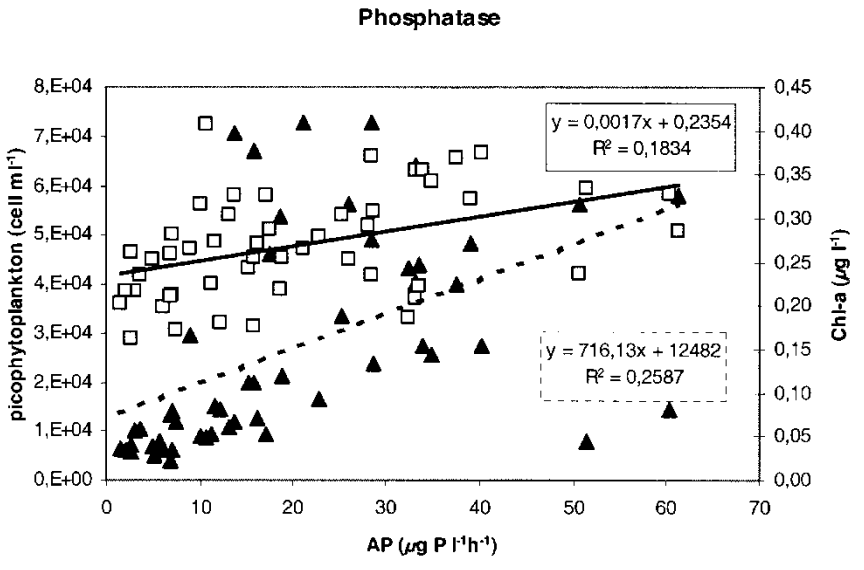

FIGURE 5 Alkaline phosphatase vs. picophytoplankton (▲) and total chlorophyll-*a* (□) ($n = 53$, $P < 0.01$).

ANOVA showed significant variations between the two days in both light and dark periods ($F = 12.44$, $n = 27$, $P < 0.01$, $F = 4.36$, $n = 23$, $P < 0.05$, respectively). The highest vertical variations were observed under the DCM layer (C.V. = 39.8%).

PON concentrations showed a higher heterogeneity than POC concentrations, both daily and in the vertical distribution. The values ranged between 2.60 and 21.0 $\mu g\, l^{-1}$, with wide time variations (C.V. = 45%); variations were also observed along the water column (mean C.V. = 35.1%). ANOVA showed a significant variation between the first and second day, only during dark periods ($F = 6.62$, $n = 23$, $P < 0.05$).

The contribution of bacterial biomass (BB) to particulate carbon was on average 7.63% (ranging from 6.12% to 11.15%); phytoplankton biomass (PhB) represented 21.47% (from 14.16 to 33.47%) of total POC content; BB was always lower than the autotrophic biomass; however, it was significantly related to POC ($r = 0.34$, $n = 56$, $P < 0.05$). The minimum percentage of living biomass (PhB + BB) on POC was reached at 22.35 and 19.42 on the two days, respectively, showing a decreasing trend in the evening that was not observed separately (Fig. 6).

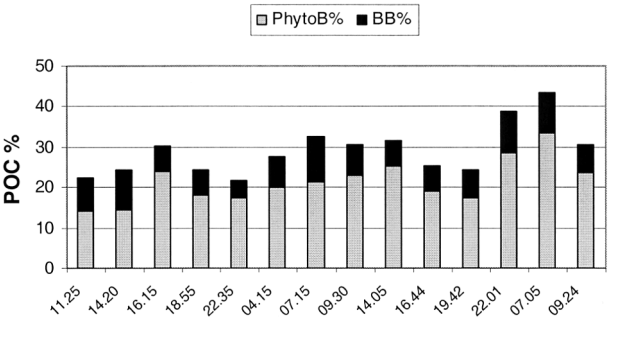

FIGURE 6 Living biomass (phyto- and bacterioplankton biomass) percentage contribution to POC during the time series.

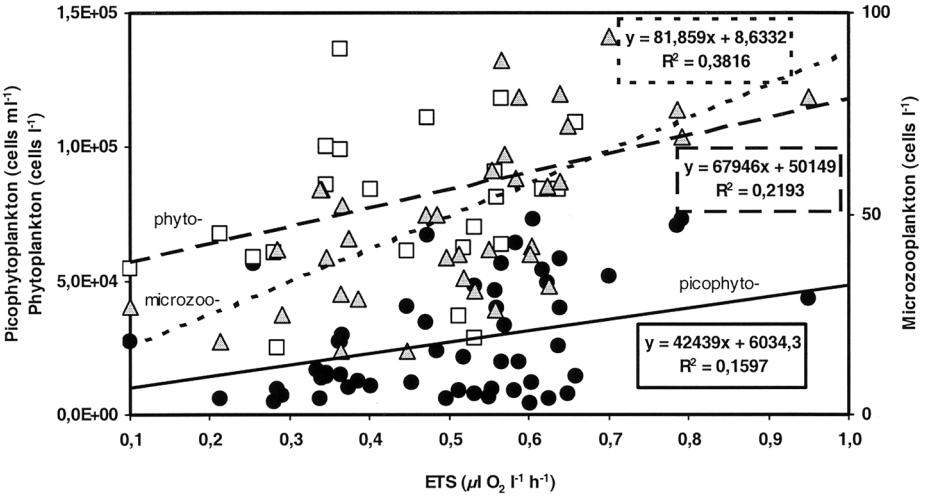

FIGURE 7 Respiration (ETS) vs. picophyto- (●) phyto- (□) and microzooplankton (▲) abundances.

3.7 Respiration

The mean ETS activity was 0.52 ± 0.13 µl $O_2\, l^{-1}\, h^{-1}$, with the lowest rates generally registered in the layer below the DCM. Respiratory activity showed significant differences between the two consecutive days, only in the dark period ($F = 11.11$, $n = 23$, $P < 0.01$), while slight differences were observed along the water column (C.V. = 37.5%; Tab. I).

Microplankton respiration showed significant correlations with the physical and chemical parameters (temperature, $r = 0.35$, $n = 54$, $P < 0.01$; oxygen, $r = -0.29$, $n = 54$, $P < 0.05$), as well as with picophytoplankton, phytoplankton and microozooplankton (Fig. 7). Its negative correlations with fluorescence ($r = -0.27$, $n = 54$, $P < 0.05$) and with oxygen indicated the dominance of the autotrophic process over consumption in the DCM.

4 DISCUSSION AND CONCLUSIONS

4.1 Variations in the Microbial Population

A high variability was observed in the different populations studied with no evident daily cycle. The various microbial fractions, which showed different physiological states, played an important role in the productive processes within the DCM. It was difficult to explain the observed variability in microbial populations by changes in light and dark period, because external input modified the distribution of community. Such variability probably reflected different sources of carbon and/or nutrients due to the changing currents.

However, the total phyto-biomass showed slight variations with no significant daily differences by ANOVA. Significant differences were observed only in the microplankton fraction of Chl-*a* ($F = 6.24$, $n = 22$, $P < 0.05$) suggesting that the major changes happened during the two days of the experiment and that they were not due to the day–night cycles. Moreover, a succession of different autotrophic fractions occurred (Decembrini *et al.*, 2004).

As regards the microscopical analysis of phytoplankton populations, being limited to the first day of sampling, it cannot provide a clear picture of the changing hydrological

conditions. However, diatoms seemed to be responsible for the DCM. They were characterized by a high biodiversity and were represented mainly by nano- (*Chaetoceros* sp.) and micro-sized species (*Leptocylindrus danicus* and *Pseudo-nitzschia pseudodelicatissima*). Also, nanophytoflagellates contributed significantly to the phytoplankton community, but they showed a rather homogeneous distribution within the water column.

The smaller autotrophic fractions (pico- and nano-) were well correlated, and their contribution in oligotrophic ecosystems is well known with regard to both biomass and productivity, in place of large phytoplankton (Maranon *et al.*, 2001). The predominance of these smaller autotrophic components and highly significant correlations of picophytoplankton with chemical, physical and biological parameters confirmed their fundamental role in this oligotrophic ecosystem.

The prevalence of the autotrophic component in the DCM was confirmed by the chlorophyll biomass that always exceeded the bacterial biomass. This agrees with Pedrós Alió *et al.* (1999), who showed that BB is lower than PhB in the DCM, but not at the surface layer. The average BB/PhB ratio (0.37 ± 0.13) was higher than the typical range (<0.2) reported by Ducklow and Carlson (1992) for coastal environments. This ratio indicated the relevance of the picoplankton system in the DCM. The ratio between living biomass (BB + PhB) and POC showed that more than 50% of POC was in the detritus form. Our results showed lower values than those regarding the oceanic euphotic zone found by Cho and Azam (1990), showing a ratio that reached about 50% of POC. A high percentage of bacterial biomass (20%) was also found in the north-west Mediterranean Sea by Van Wambeke *et al.* (2001).

Diel variations in bacterial abundance and activity found in oligotrophic zones showed the highest bacterial abundance early in the morning (Fuhrman *et al.*, 1985; Gasol *et al.*, 1988; Kuipers *et al.*, 2000). In coastal eutrophic zones, these bacterial abundances were not coupled with diel cycles and were also affected by the distance from the coast and the season (Puddu *et al.*, 2000). Our results demonstrated differences in bacterial abundance between the first and the second day, which were not related to the light–dark cycle and were not coupled to the DCM.

The analyses of viable/active fractions allowed a more accurate estimate of the effective abundance of the viable bacterial biomasses and of their activity in the environment. NuCC percentages were similar to those reported by Karner and Fuhrman (1997), while higher percentages (30–50%) were observed in the north-west Mediterranean by Gasol *et al.* (1998). The active/living fraction of bacterioplankton did not show a clear decreasing trend with respect to depth and relevant time differences. This is probably due to not significant variations within the short depth/time ranges considered. Previously, Gasol *et al.* (1998) did not find any diel cycle at the coastal station of the north-west Mediterranean. On the contrary, a diel cycle with maximum NuCC values during the day at the surface and DCM layers occurred at the offshore stations. Other authors observed a diel variation in the NuCC abundance with a minimum concentration at midday (Hagström *et al.*, 2001).

The role of microzooplanktonic protozoa in the microbial loop in recent years has been re-evaluated because of the transfer of material and energy from nano- and picoplankton to upper trophic levels. In this study, the microzooplankton abundance was low and showed the prevalence of tintinnid fraction with no clear daily cycle. Tintinnid densities were in the same order as those observed by Sparla and Guglielmo (1992) in the Straits of Messina, showing a distribution related to hydrodynamic circulation of water masses. A higher abundance ($0–30.5 \times 10^3$) with a low variability was observed in the Naples Gulf, and this was related to phytoplankton biomass and food availability by Modigh and Castaldo (2002).

4.2 Microbial Activities

Microbial activities showed a higher variability than total bacterial abundance, showing wide variations during sampling times for both LAP and β-GLU. As observed in other coastal environments (La Ferla *et al.*, 2002; Zaccone and Caruso, 2002), the variations according to depth were also important, with a decreasing trend from the surface to the bottom. Time variations were observed in the North Adriatic Sea during the winter (Puddu *et al.*, 2000), and in the north-west Mediterranean (Van Wambeke *et al.*, 2001), but they were in a lower range than those reported in this study.

The microbial community of DCM reacts quickly to changing environments, as observed at 14.20 on the first day, increasing the biomass and the potential of the enzymatic spectrum, as indicated by the important relationship between hydrolysis and organic matter available.

The organic carbon decomposed by bacterial hydrolytic enzymes may be an index of bioavailable resources for bacteria nutrition, even if we take into account that they are potential data. An attempt to evaluate the C flux through the bacterial compartment was calculated by both LAP and β-GLU as the percentage ratio between POC and the potential hydrolysis. We estimate that in the DCM, on average 25.5% of particulated carbon per day could be hydrolysed and returned to the environment supporting microbial growth. However, not all mobilized carbon can be assimilated immediately by bacteria; other factors such as environmental characteristics or changes in the physiological status of cells can affect this process (Hoppe *et al.*, 2002).

The peak of LAP and β-GLU occurred in the change in direction of the main current, carrying a different water mass from the north area. The peak of total bacteria and POC was also observed, so we calculated a consistent percentage of POC degradation in the first day at 14.20 (72.7%); another maximum of POC degradation was calculated at 7.05, 11 Oct. 2001 (79.8%). A corresponding peak of nitrate at the beginning of Phase I and IV in the southward current confirmed our hypothesis (Decembrini *et al.*, 2004).

In our study, the different fractions of planktonic organisms contributed to enzymatic activities and respiration, as indicated by the regression coefficients (LAP was related to Ciliophora and Tintinnida). In fact, ectoenzymes can be produced by a large number of micro-organisms such as zooplankton and nanoflagellates, although their main producers are algae and bacteria (Bockdansky *et al.*, 1995; Hoppe *et al.*, 2002).

The synthesis of AP is recognized as playing an important role in the regeneration of P from organic P esters. During the first day, the concentrations of inorganic P and nitrate were sufficient to sustain the production. We observed an increase in AP in the second day, early in the morning and at 14.05, when inorganic P was probably consumed (less than 0.15 $\mu g\, l^{-1}$). Sala *et al.* (2001) proposed the use of the AP/LAP ratio to assess the nutrient limitation in the microbial population. According to this approach, in the DCM, a rapid recycling of phosphorus with respect to C and N was observed. However, the mechanisms of regeneration may be different for N and P: in fact, while hydrolysis of proteins released amino acids, which follow different routes before complete mineralization, AP activity produced an orthophosphate molecule.

In the euphotic zone, AP activity may originate from algae, free-living and attached bacteria, and protozoa. In different marine environments, AP covers a wide range and increases in regions with a high degree of eutrophication, with an abundance of bacteria and phytoplankton and a low concentration of inorganic P (Hoppe, 2003). However, the regulation mechanisms of AP stimulation by inorganic P are different in algae and bacteria; while algae produced AP in P limited conditions (Chròst *et al.*, 1989; Vrba *et al.*, 1995), the mechanism was not so close for bacteria. The bacteria can produce AP also when P is abundant to

regenerate organic C substrates, which are also final products of phosphoester hydrolysis (Hoppe and Ullrich, 1999; Hoppe, 2003).

In contrast to LAP, AP did not correlate with total bacteria, thus supporting the hypothesis that the other fraction in the DCM significantly contributes to the P release for its metabolism, playing a predominant role in the AP production. The significant correlation with chlorophyll *a* suggested that AP was mainly produced by autotrophic organisms. About 25% of AP variance may be explained by picophytoplankton variations (Fig. 5), suggesting that picophytoplankton was significantly involved in enzyme production at the DCM, as observed in other environments (Li *et al.*, 1998).

Finally, respiration proved to be a key parameter owing to several correlations with the biotic and abiotic compartments. In fact, along the water column, the different fraction of planktonic organisms contributed in a synergic way to respiration. In the DCM layer, the negative correlation between R and fluorescence and oxygen showed that the oxygen generated during the photosynthetic period was only partially reduced during darkness, when higher respiratory processes were presumed. This suggested that the productive and consumptive processes were uncoupled. Such evidence highlighted that in areas affected by a DCM, lower respiration tenors must be supposed. Two factors seemed to affect the respiration along the water column: the different 'community consortia' existing in the column and the physiological state of the organisms occurring in the layers.

In conclusion, micro-organisms played an active role in the environment, displaying a different distribution in the DCM with a specific location. Their activities were due to different fractions: total bacterioplankton contributed significantly to enzymatic degradation of organic carbon (by production of β-GLU, LAP), with peaks related to changes in the main water current, while the autotrophic biomass, particularly the picophytoplankton fraction, contributed significantly to the high values of AP, suggesting rapid inorganic P regeneration in the DCM.

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