



## Diel variations in particle stocks in the oligotrophic waters of the Ionian Sea (Mediterranean)

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### ABSTRACT

In order to assess the factors responsible for the variability in particle attenuation of light at a daily scale in the oligotrophic waters of the Ionian Sea (Eastern Mediterranean), optical and biogeochemical measurements were collected at high spatial and temporal frequency during late summer 1999. Over 5 days, repeated profiles were conducted in situ every 3 h using a spectral absorption–attenuation meter at 9 wavelengths (WETLabs ac-9) in a profiling mode, and were accompanied by a series of discrete biogeochemical measurements including particle size distribution and concentration, and flow cytometric determination of picophytoplankton and heterotrophic bacteria abundances. Temporal variations in the vertical distribution of the particle attenuation coefficient and particle concentration displayed clear daily oscillations, with an increase during daytime counter-balanced by a similar decrease at night. For the first time in in situ conditions, diel changes in the particle size were also evidenced through discrete measurements of the particle size distribution and in situ continuous ac-9 profiles of the spectral characteristics of particle attenuation coefficient. The diel variability in the concentration and size of the particle stock, and its impact on bulk particle attenuation, is discussed in relation to the composition of the autotrophic assemblage, the balance between algal and non-algal particles and the specific rhythms in biological processes. In Ionian Sea waters, heterotrophic bacteria appear to play a major role in the diel cycle in particle attenuation.

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### 1. Introduction

Oligotrophic areas account for a large fraction of the world ocean and extend over various spatial and temporal scales: from large sub-tropical oceanic gyres, permanently oligotrophic, to temperate areas, where oligotrophic conditions can be encountered on a seasonal basis. In these oligotrophic systems, the main source of biophysical forcing is the day–night cycle in light intensity. Episodic events such as internal waves or wind gusts can also be important. The effect of the diel cycle of light intensity on in-water components has been reported in a variety of biogeochemical and optical quantities (e.g., microorganisms abundance, particle attenuation and scattering, chlorophyll fluorescence) in various regions of the

world ocean: from the Pacific (Siegel et al., 1989; Claustre et al., 1999; Binder and DuRand, 2002; Claustre et al., 2007; and references therein), to the north Atlantic (Gardner et al., 1993; Gardner et al., 1995; Stramska et al., 1995; Marra, 1997) and the Arabian sea (Kinkade et al., 1999; Gardner et al., 1999). Fewer studies examined diel patterns in the Mediterranean (e.g., microorganisms abundance; Perez et al., 2000; Jacquet et al., 2002). In regions of no winter mixing, the diel variability is often more important than the variability at the weekly scale, or even at the annual scale (Stramska et al., 1995). This scale of variability is often neglected in sampling strategies and in the interpretation of optical and biogeochemical data acquired in oligotrophic systems (in situ or from remote sensing), and usually considered as biological “noise”. To fully understand biogeochemical processes, it is essential to examine the extent of variability on the diel scale. The diel variability became recently more accessible with the development of moorings equipped with sensors for the description of various key bio-optical quantities (e.g., fluorescence, attenuation and

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absorption coefficients; Chang and Dickey 2001; Dickey, 2003; Chang et al., 2006) with a high temporal resolution.

The variability of the various properties characterizing the particle stock, such as concentration or size, is particularly affected by diel changes in light intensity. This stock encompasses different types of material, ranging from phytoplankton (directly dependent on the diel cycle in light intensity) to heterotrophs (bacteria, ciliates and flagellates) and detritus. The daily patterns in bulk particle attenuation ( $c_p$ ) observed in most oceanic oligotrophic regimes are driven by changes in: i) the composition and abundance of the particle assemblage and ii) the optical properties of the individual particles which form this assemblage. The diel cycle in  $c_p$  has often been associated with the daytime accumulation and nighttime removal of particles, and in particular of algal cells. Subsequently, attempts were made to estimate primary or community production (and associated growth rates), by converting the daily increase in  $c_p$  into daily accumulation of particulate organic carbon (POC) (see Binder and DuRand, 2002 for a detailed review). However, these conversions rely on various assumptions, including a constant conversion factor between  $c_p$  and POC (carbon-specific particle attenuation coefficient,  $c^*_c$ ), an assumption which has been contradicted by various laboratory observations on phytoplankton cultures (Stramski and Reynolds, 1993; Stramski et al., 1995; DuRand and Olson, 1998).

The present study examines the diel variations in bulk spectral  $c_p$  together with changes in particle numerical concentration, size and composition (i.e., numerical abundance of various phytoplankton and heterotrophic bacteria). The WETLabs ac-9, an in situ optical device, was used in vertical profiling mode for measuring the absorption and attenuation coefficients at 9 wavelengths, together with the simultaneous sampling of a set of discrete biogeochemical quantities. The

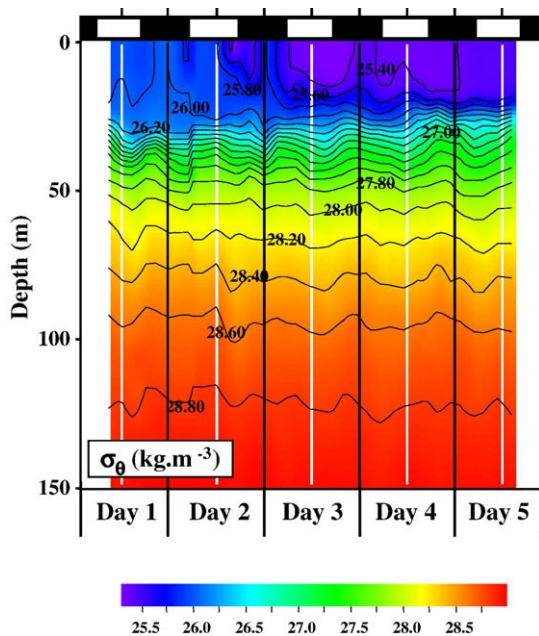


Fig. 1. Temporal evolution of the density excess at MIO (Ionian Sea) between 0 and 150 m during 5 days (20–21 September 1999).

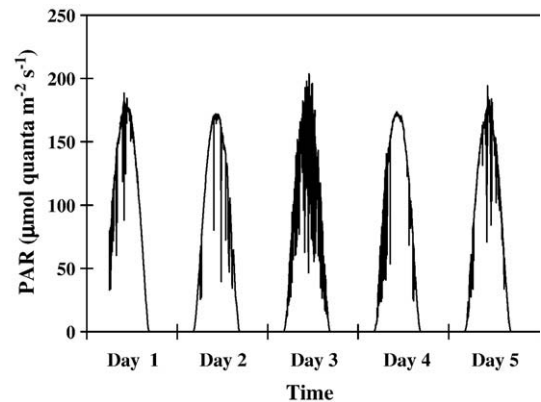


Fig. 2. Temporal evolution of PAR (photosynthetically available radiation) at MIO (Ionian Sea) between 0 and 150 m during 5 days.

sampling was realized with a high temporal frequency (every 3 h) during 5 days at a fixed site in an ultra-oligotrophic environment in the eastern Mediterranean (MIO site, Chl  $a < 0.3 \text{ mg m}^{-3}$  at the chlorophyll maximum; Oubelkheir et al., 2005). A detailed description of the optical and biogeochemical properties of the water column at the MIO site is given in Oubelkheir et al. (2005). The aim of this study was to determine how the bulk spectral particle attenuation varied in the surface layer in a typical oligotrophic system on a short temporal scale (from hours to days), and to what extent changes in the particle assemblage concentration, size and composition affect the observed patterns.

## 2. Materials and methods

### 2.1. Sampled sites

Hydrographic, bio-optical, and biogeochemical data were simultaneously acquired in the Moroccan upwelling and in the Mediterranean in late summer (from 4 September to 4 October 1999), during the PROSOPE cruise onboard R/V Thalassa. The present study focuses on the results obtained in the Ionian Sea at the MIO site (see Fig. 1 in Oubelkheir et al., 2005), which was sampled with a high temporal frequency (every 3 h) between the 20 September and 24 September 1999. The full sets of measurements performed during the PROSOPE cruise are described in detail in Oubelkheir et al. (2005). In what follows, we briefly recall the measurements which are relevant for the present study.

### 2.2. Optical data acquisition

A WETLabs ac-9 was used to measure spectral attenuation and absorption coefficients at nine wavelengths (412, 440, 488, 510, 532, 555, 630, 676, and 715 nm). The ac-9 was attached to a Seabird carousel, equipped with 21 Niskin sampling bottles (12-L) and a Seabird 911-Plus CTD. This package was deployed between 0 and 400 m. Profiles were conducted with a vertical resolution of ca. 0.1 m. The ac-9 was calibrated twice onboard with optically-pure water (MilliQ® A10 system) to quantify instrumental offsets, before and after the 5 days high temporal frequency measurements period. When corrected for these offsets, measured absorption ( $a(\lambda)$ )

and attenuation ( $c(\lambda)$ ) coefficients exclude the contribution by pure water. Corrections for in situ temperature and salinity effects on the optical properties of the water were applied according to Pegau et al. (1997). Correction for incomplete recovery of the scattered light in the ac-9 absorption tube was performed by subtracting  $a(715)$  from  $a(\lambda)$  (Zaneveld et al., 1994) (as in Oubelkheir et al., 2005). Finally, ac-9 data were averaged over 1-m intervals. Data analysis revealed an instrumental drift over time (as already reported by Twardowski et al., 1999), monitored from the temporal evolution of  $a(\lambda)$  and  $c(\lambda)$  at 400 m in the Mediterranean. Absorption and attenuation data were thus recalibrated using an original method described in Oubelkheir et al. (2005).

Colored dissolved organic matter (CDOM) absorption is estimated through the partition of the spectral total absorption coefficient ( $a(\lambda)$ ) into phytoplankton and colored detrital material ( $CDM = CDOM + \text{non-algal particles}$ ) contributions, following a method described in detail in Oubelkheir et al. (2007). This partitioning method is adapted from Bricaud and Stramski (1990) for application to total absorption spectra (instead of particulate absorption in Bricaud and Stramski, 1990), and has been validated over a wide range of trophic conditions (Oubelkheir et al., 2007). At the MIO site, non-algal particle contribution in CDM is minor (<8%) so that particle attenuation coefficient ( $c_p(\lambda)$ ) can be computed as the difference between measured attenuation ( $c(\lambda)$ ) and computed CDM absorption ( $a_{CDM}(\lambda) \sim a_{CDOM}(\lambda)$ ) coefficients. The spectral dependency of  $c_p(\lambda)$  is subsequently modeled according to an hyperbolic function (Boss et al., 2001; Van de Hulst 1957; and references therein) using a non-linear regression method:

$$c_p(\lambda) = \alpha \lambda^{-\gamma} \quad (1)$$

where  $\gamma$  is the slope of the spectral dependency and  $\alpha$  is a scaling factor.

A PAR (Photosynthetically Available Radiation, i.e., the 400–700 nm band) sensor with a spherical collector was mounted on the ship's superstructure to monitor the incident solar irradiation for the spectral PAR domain.

### 2.3. Discrete data acquisition

Water samples were regularly collected for various discrete biogeochemical measurements, as described in detail in Oubelkheir et al. (2005). Autotrophic picoplankton, heterotrophic bacteria and virus enumeration was performed on seawater samples fixed with 0.1% glutaraldehyde, using a flow cytometer (FACSort, Becton Dickinson) and following the protocols described in Marie et al. (1999, 1997). Particle size distributions were measured using a HIAC optical counter (Royco–Pacific Scientific). The measurement is based on the light blockage principle. Further description of the counter principle and functioning is given in Bernard et al. (1996).

The various parameters used in this study are defined here. If  $n_i$  is the number of particles counted in the size class  $i$ ,  $N = \sum_{i=1}^m n_i$  is the total number of particles counted in the sample volume  $V$  between the size classes  $i=1$  and  $i=m$ . In the present study, the lower and upper size limits are respectively 1.6 and 50  $\mu\text{m}$ , and counts are distributed within  $m=85$  log-normal size classes. The Junge exponent ( $j$ ) is estimated from the slope of the linear regression on the log-transformed particle densities in the 1.6–2.6  $\mu\text{m}$  size range. The computation of  $j$  on this restricted range (11 size classes) was constrained by an instrumental artifact appearing around 2.6  $\mu\text{m}$  (resulting from an uncertainty in the calibration curve of the HIAC particle counter around this domain). More details on the measurements of the various quantities described here can be found in Oubelkheir et al. (2005). The sampling depths were ~5, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150 m.

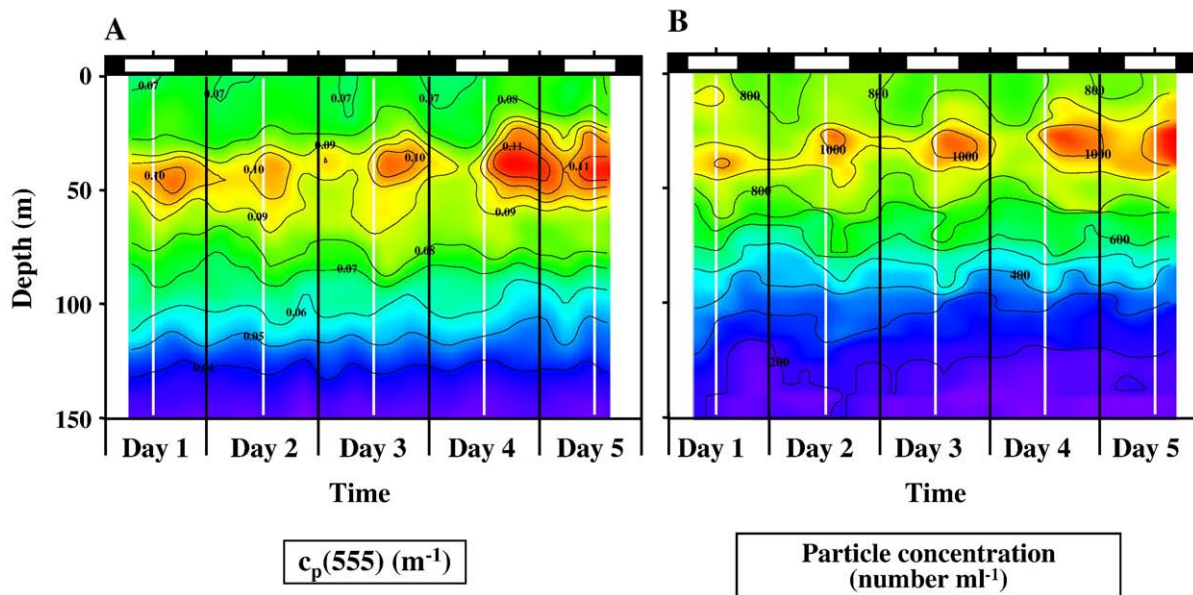


Fig. 3. Temporal evolution of (A) particle attenuation ( $c_p(555)$ ) measured using an ac-9, (B) particle numerical concentration measured by a HIAC particle counter at MIO (Ionian Sea) between 0 and 150 m during 5 days.

### 3. Results

#### 3.1. Environmental conditions

The density profile was characterized by a pronounced pycnocline located at ~30 m (Fig. 1). A regular “longer-term” decrease in density was observed from day 1 to day 5 ( $\sigma_\theta$  decreased from 25.97 to 25.40 in the surface layer, respectively). Surface waters were nutrient depleted (down to 80 m for  $\text{NO}_3$ , respectively; Raimbault, P., pers. comm.). The euphotic depth (i.e., the depth where PAR was reduced to 1% of its surface value) was on average 82 m (Hooker, S., pers. comm.). The temporal evolution of PAR at the surface (in the air) is given in Fig. 2. Sky conditions were cloudy on day 3 and relatively clear over the rest of the study period.

#### 3.2. Particle attenuation

The particle attenuation ( $c_p$ ) time series showed clear daily oscillations, with a minimum near sunrise (~04h30, local time) and a maximum near sunset (~16h30), with the largest amplitude corresponding to the depth of the  $c_p$  maximum (Fig. 3A). The  $c_p$  maximum was oscillating between 40 and 45 m, with a corresponding daily percent increase comprised between 5% and 21% (day 2 and 4, respectively; the percent increase was computed relatively to the  $c_p$  minimum). Superimposed on these diel patterns, a “longer-term” increase was also noticed over the 5 days of the sampling period and equaled ~5% from day 1 to day 4 (at sunset, the  $c_p$  maximum equaled  $0.113 \text{ m}^{-1}$  and  $0.118 \text{ m}^{-1}$ , respectively).

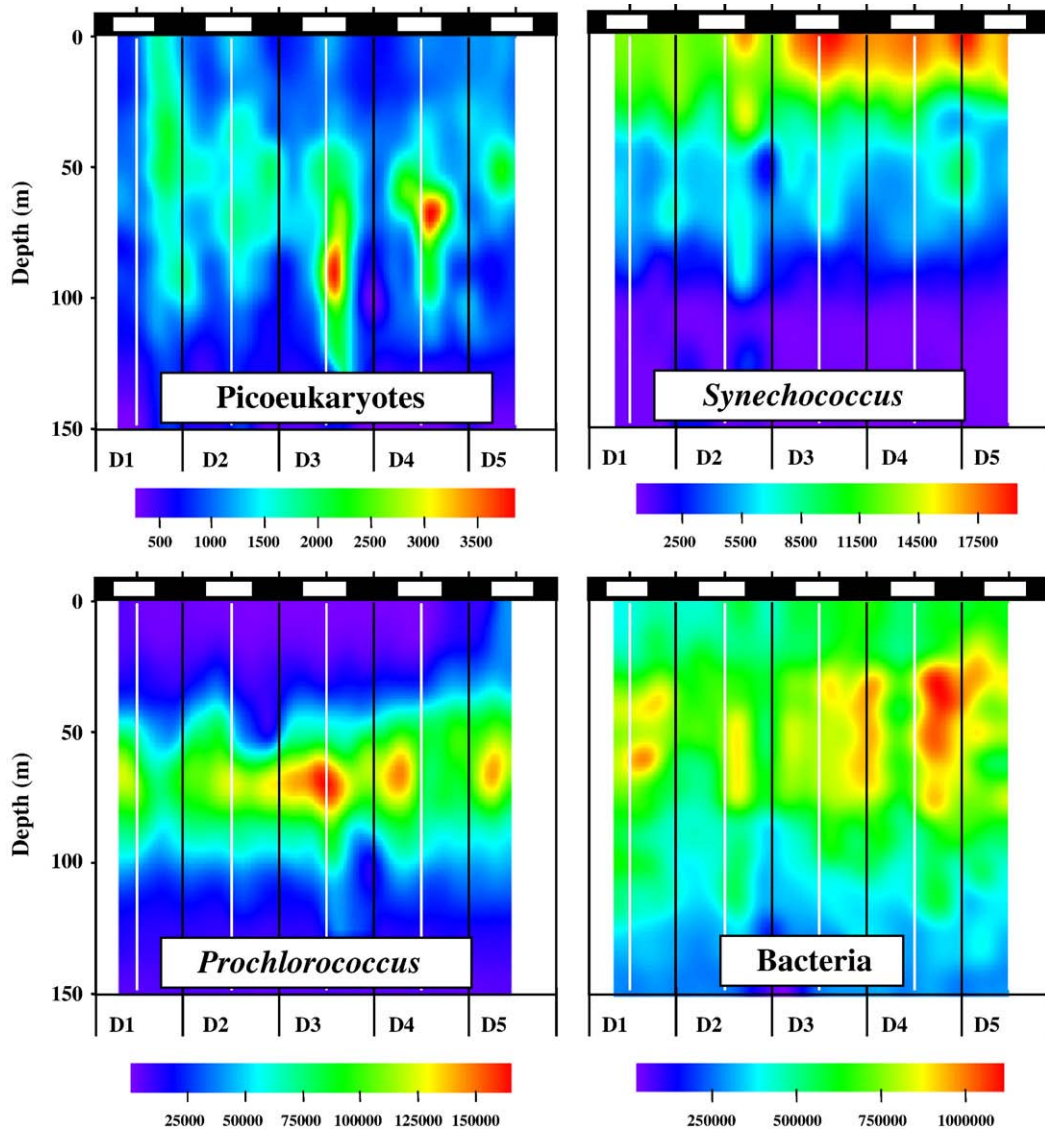


Fig. 4. Temporal evolution of the numerical concentration (cells per mL) of various microorganisms (picoeukaryotes, *Synechococcus*, *Prochlorococcus* and heterotrophic bacteria) acquired using a flow cytometer at MIO (Ionian Sea) between 0 and 150 m during 5 days.

### 3.3. Particle numerical concentration (bulk material, phytoplankton and heterotrophic bacteria)

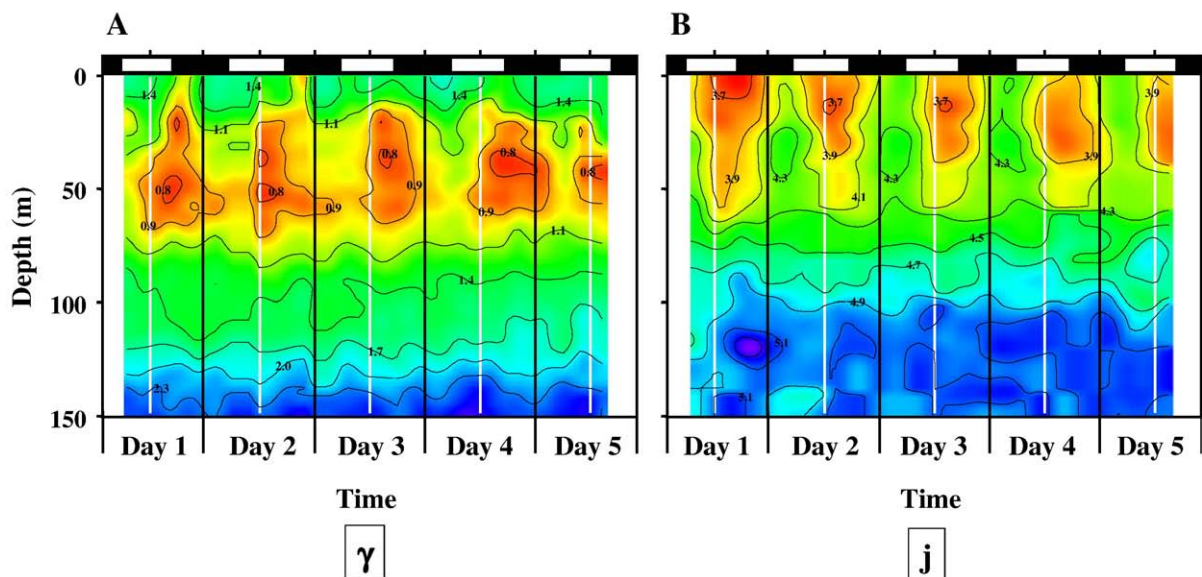
The bulk particle numerical concentration showed daily oscillations similar to the ones observed in  $c_p$  (Fig. 3B), with an increase from sunrise to sunset counter-balanced by a decrease of similar amplitude during nighttime. The largest amplitude in day–night oscillations was noticed at the depth of the  $c_p$  maximum (~30 m, corresponding to the depth where the discrete samples were taken, see Materials and methods) with a daytime percent increase comprised between 6% and 8% (on day 2 and 4, respectively). A “longer-term” increase (from day 1 to day 5) was noticed, as in  $c_p$ , and was equivalent to ~20%.

The phytoplankton assemblage was dominated by *Prochlorococcus*, *Synechococcus* and picoeukaryotes, which are typical of oligotrophic systems (e.g., *Vaulot and Marie, 1999*). Their numerical abundance was characterized by different spatio-temporal distributions (Fig. 4, cells per mL) as described below. While *Synechococcus* abundance was maximal in the surface layer, picoeukaryotes were usually more abundant in the 50–90 m layer (although pretty well mixed from the surface down to 100 m on day 1) and *Prochlorococcus* at ~65 m. No clear diel oscillations were observed in their abundance time series (despite two maxima in picoeukaryotes abundance just after noon on day 3 and day 4). Interestingly, *Synechococcus* abundance showed a tendency to increase over the 5 days of the sampling in the surface layer, coincidentally to the surface decrease in the surface density (Fig. 1). By contrast, the abundance in heterotrophic bacteria cells showed daily oscillations with maximal amplitudes at the depth of the abundance maximum (at ~40 m, daytime percent increase equal to up to 90%), and a “longer-term” increase over 5 days (6% increase of the maximum value). Interestingly, these patterns seen in heterotrophic bacteria cell variability were similar to the ones observed in  $c_p$  and the bulk particle numerical concentration.

### 3.4. Particle size

A concurrent study performed at MIO site evidenced a linear relationship between the Junge exponent of the particle size distribution ( $j$ ) and the spectral slope of the particle attenuation coefficient (characterized by  $\gamma$ ) (*Oubelkheir et al., 2005*; their Fig. 3). This relationship was of the type:  $j = \gamma + 3$ , in agreement with what is predicted by theoretical studies based on Mie computations (*Van de Hulst, 1957*; *Boss et al., 2001*; and references therein). In the upper water column, the lower values in  $\gamma$  and  $j$  were indicative of a particle assemblage dominated by larger particles, while in the deeper layer, the increase in  $\gamma$  and  $j$  showed a decrease in particle size (Fig. 5). Temporally, measured ( $j$ ) and optically-derived ( $\gamma$ ) Junge exponents both presented clear daily oscillations, with a daytime increase in relative particle size ( $j$  decreases from ~4.1 at sunrise to 3.7 at sunset; and  $\gamma$  from ~1.1 to 0.8) balanced by a relative decrease of similar amplitude during nighttime (increase in  $\gamma$  and  $j$ ). However, the amplitude of day–night variations was maximal in different layers for both estimators of the particle size: at ~40–50 m for  $\gamma$  and in the near surface layer for  $j$ .

The comparison between quantities measured on discrete samples ( $N/V$  and  $j$ ) or using in situ optical instruments ( $c_p$  and  $\gamma$ ) shows differences in the spatio-temporal distributions of i)  $c_p$  and the particle numerical concentration and ii)  $\gamma$  and  $j$ . These differences are probably due to various factors: first, optical sensors used in a profiling mode capture better the fine scale vertical variability (see sampled depths for discrete samples in the material and methods) and, second, the different techniques are resolving i) different portions of the size distribution and ii) different aspects of the particles. As an example,  $c_p$  and  $\gamma$  integrate the contribution by most particles present in the water; assuming a Junge-type size distribution with an exponent close to 4, the ac-9 views particles mostly in the 0.5–10  $\mu\text{m}$  range. By contrast, the



**Fig. 5.** Temporal evolution of (A) the spectral slope of the particle attenuation coefficient ( $\gamma$ , computed from ac-9 measurements and derived  $c_p$ ) and (B) the particle size distribution Junge exponent ( $j$ , calculated from particle counter measurements), at MIO (Ionian Sea) between 0 and 150 m during 5 days. Both scales are in inverse colors.

particle counter doesn't "count" the particles smaller than 1.6  $\mu\text{m}$  (the lower size limit of the counter), and  $j$  was computed here on a restricted size range (1.6–2.6  $\mu\text{m}$ ), which excludes part of the heterotrophs (size range of various heterotrophs, bacteria: 0.2–1  $\mu\text{m}$ , Ducklow, 1986, flagellates:  $\sim 3 \mu\text{m}$ , and ciliates:  $\sim 12.5 \mu\text{m}$ , Morel and Ahn, 1991). This Junge exponent is assumed representative of the size distribution of all the particles seen by the ac-9. However, some specificities in the dynamic of the size distribution might exist in certain size classes, so that the Junge exponent computed in this restricted size range might not be representative of all particles.

## 4. Discussion

### 4.1. Factors responsible for the diel cycle in $c_p$

A large diversity of particles occurs in oceanic waters, from various phytoplankton and heterotrophs such as bacteria, flagellates and ciliates, to various types of detritus. The factors responsible for diel patterns in the bulk attenuation of light by particles are closely inter-related and are the result of either changes in the properties of individual particles (numerical concentration, size, refractive index, shape, internal structure) and/or of external environmental or biological forcing on these various particles (Binder and DuRand, 2002; Claustre et al., 1999; and references therein). The bulk particle attenuation coefficient ( $c_p$ ) is equal to the product between the numerical concentration of all particles present in a given water parcel ( $N/V$ ), their geometric cross section ( $s_g$ ) and their efficiency factor for attenuation ( $Q_c$ , dimensionless) (Morel and Bricaud, 1986; Van de Hulst, 1957) such as:

$$c_p = \frac{N}{V} \times s_g \times Q_c \quad (2)$$

The particle attenuation cross section ( $\sigma_c$ ) is defined as the product between  $s_g$  and  $Q_c$ .  $Q_c$  varies as a function of the refractive index (real and imaginary parts) and the size of the particles. For particles smaller than a few microns,  $Q_c$  decreases with decreasing size, while for particles larger than a few microns,  $Q_c$  oscillates around 2 so that the critical parameter affecting  $c_p$  will be  $s_g$ . Factors on the right hand side of Eq. (2), including total particle number, the geometric cross section for each particle, and the refractive index of each particle, can vary with time and lead to changes in  $c_p$ . Changes in the shape (can affect  $s_g$  and  $Q_c$ ) and internal structure (can affect  $Q_c$ ) of the particles can also occur (e.g., as a result of growth and cellular division) and should ideally be taken into account for a correct interpretation of the (diel) variability in the  $c_p$  signal.

Photosynthetic production is one of the main sources of particulate material and  $c_p$  in oligotrophic waters (Siegel et al., 1989; Walsh et al., 1995; Durand and Olson, 1996). During daytime, phytoplanktonic cells fix inorganic carbon, which leads to an increase of phytoplankton mean diameter and index of refraction (through an increase of their intracellular carbon concentration, Stramski, 1999). Both scattering and attenuation cross sections thus increase. Studies performed in the laboratory on various phytoplankton species (*Synechococcus*, Stramski et al., 1995; *Nannochloris* sp., DuRand and Olson, 1998; *Thalassiosira pseudonana*, Stramski and Reynolds, 1993; *Prochlorococcus*, Claustre et al., 2002) showed that the particle concentration is not necessarily the main factor responsible for the diel variability in the phytoplankton particle scattering and attenuation signal; variations in the refractive index and the diameter of the cells during the cellular cycle are also important. As a result of photosynthesis and associated growth, the attenuation cross section of phytoplankton cells ( $\sigma_c$ ) was shown to increase during daytime by 72% to 184% (relative to morning value and at 660 nm; Claustre et al., 2002 their table 2, and references therein). Loss terms are less known than source terms, and encompass cellular division and respiration, grazing pressure, viral lysis, aggregation, sedimentation and nocturnal convection processes (Binder and DuRand, 2002 and references therein). During nighttime, the phytoplankton cells divide into smaller cells with lower carbon content per cell, as a result of an uptake of water when dividing or loss through respiration (DuRand and Olson, 1998). The effect of a simultaneous increase of the cells numerical concentration and decrease in their size due to cellular division is difficult to quantify. Claustre et al. (1999) and DuRand and Olson (1996) estimated that the cellular division roughly accounted for 12 to 20% of the  $c_p$  change in the case of a phytoplankton population of very small cells with a given size dividing synchronously.

In situ conditions, the diel patterns in  $c_p$  are the integrated result of the dynamic of algal and non-algal (various heterotrophs and detritus) stocks and may vary as a result of changes in the trophic conditions (i.e., balance between algal and non-algal stocks). Some of the main questions to address are: which parameter on the right hand side of Eq. (2) (numerical concentration, size and/or refractive index), and which stocks (phytoplankton, heterotrophs or bio-detritus) are driving the observed patterns in  $c_p$  variability over the diel cycle in a given area and for a given trophic condition? In natural conditions, Vault and Marie (1999) showed that the cellular cycles of phytoplankton and heterotrophs are not in phase; despite that they are synchronous for a given phytoplanktonic population in a given area.

In Ionian Sea waters, the daytime percent increase (relative to morning value) in  $c_p$  measured at MIO (5–21%) is within the range of values reported by previous studies performed in the Pacific. These latter showed that the extent of daytime increase in  $c_p$  was variable, ranging between 2% (Siegel et al., 1989, north Pacific), 10–30% (Chung et al., 1998, equatorial Pacific), 25–50% (Claustre et al., 1999, tropical Pacific), 50–60% (Durand and Olson, 1996, equatorial Pacific), and up to 70% (Gardner et al., 1995, equatorial Pacific). At MIO, diel patterns in  $c_p$  were driven both by the concentration and the size of the particles (Figs. 3 and 5). Note that the patterns observed here in particle concentration are different to what was observed in other field studies and laboratory experiments on phytoplankton (Stramski and Reynolds, 1993; Stramski et al., 1995; Durand and Olson, 1996; Binder and DuRand, 2002; and references therein). These studies showed that particle size increased during daytime (as observed here), while particle concentration decreased (inversely to what was observed here), as a result of the balance between growth (increase in cell size) and division (decrease in cell size and increase in numerical concentration) processes. Such unexpected trend in the diel cycle of particle concentration might be due to the fact that the particle counter does not incorporate the dynamic of particles

smaller than 1.6  $\mu\text{m}$  (see above) or a fast turnover rate of the particulate material (see below).

Our results are the first to show diel variations in the size structure of the particle assemblage in situ using optical techniques in profiling mode in the water column. To the best of our knowledge, all published data showing diel changes in particle size were derived from light scattering measurements by flow cytometry (discrete samples) on algal and/or heterotrophic bacteria cells. As underlined by various authors, relating changes in flow cytometrically-measured light scattering to cell size is not straightforward and depends on the inherent optical properties of the cells (e.g., refractive index) and on the angle of measurement of the scattered light (Binder and DuRand, 2002 and references therein). Similarly, relating the spectral slope of the particulate attenuation exponent ( $\gamma$ ) to the Junge exponent of the particle size distribution ( $j$ ) also relies on various assumptions discussed elsewhere (Oubelkheir et al., 2005). However, this in situ optical approach provides a description of the variability in particle size with a higher spatial and temporal resolution than the flow cytometrically-based approach, if the relationship between  $j$  and  $\gamma$  is known over the range of conditions explored.

#### 4.2. Diel cycle in $c_p$ and the role of heterotrophic bacteria

Heterotrophic bacteria are one of the main drivers of the diel patterns in  $c_p$  in the Ionian Sea: similar spatio-temporal patterns (vertical distribution, diel patterns and “longer-term” increase over 5 days) were observed in  $c_p$ , the numerical concentration of bulk particles and the cell abundance of heterotrophic bacteria (Figs. 3 and 4). This is in contrast with most previous studies where variations in phytoplankton properties (size, refractive index, see above) accounted for most of the diel variations in  $c_p$  (Durand and Olson, 1996; Binder and DuRand, 2002 and references therein); with the exception of Chung et al. (1998) who concluded that neither phytoplankton, nor heterotrophic bacteria dynamics were sufficient to explain the observed patterns. Differences in the factors leading to diel variations in  $c_p$ , relative to other studies performed in the Pacific (see above), may arise from methodological differences, but we must definitively underline some probable regional/trophic ecosystems functioning specificities. None of the major phytoplankton components covary with  $c_p$  or heterotrophic bacteria abundance, underlying nuances in case 1 waters definition as discussed in detail in Oubelkheir et al. (2005). More detailed studies on the optical properties of various non-algal stocks (heterotrophic bacteria, flagellates, ciliates and detritus) and associated diel variations are required in the laboratory and in in situ conditions.

The diel patterns in particle size observed in the Ionian Sea (daytime increase and nighttime decrease, Fig. 5) are in agreement with the diel variations in the mean diameter of bacterial cells reported by Kuipers et al. (2000) in the subtropical Atlantic. However, the same authors underlined a peak in bacterial cells concentration during nighttime, while our results suggest the highest bacterial cells concentration at the end of the day. Interestingly, Fuhrmann et al. (1985) observed a morning increase in bacterial cells concentration followed by an afternoon and evening decrease attributed to grazing by microflagellates. The concurrent daytime increase in particle

concentration and size observed here suggests a rapid turnover rate (division rate) of heterotrophic bacterial cells. This hypothesis requires further investigations by performing independent estimations of bacterial growth and division rates. More studies are also required on the diel patterns in the grazing pressure on phytoplankton (by zooplankton) and on bacteria (by ciliates, flagellates) (Fuhrmann et al., 1985 and references therein), in order to acquire a proper understanding of the factors responsible for the diel variability in  $c_p$ .

Interestingly, most authors attributed the diel variations in  $c_p$  to phytoplankton (Binder and DuRand, 2002 and references therein), but they also underlined a major contribution of non-algal stocks (mainly detrital) to the  $c_p$  signal. Such an observation has been corroborated at MIO site by a  $c_p$  budget presented in Oubelkheir et al. (2005), which showed that detritus accounted for up to 60% of  $c_p$ . These budgets rely on various assumptions (see Binder and DuRand, 2002 and references therein) and are thus subject to uncertainties (see discussion in Oubelkheir et al., 2005). One of these limitations is the lack of knowledge of the individual optical properties of non-algal particles (bacteria, ciliates, flagellates and detritus). A particle stock can be the main contributor to the absolute magnitude of  $c_p$  but account for a small or negligible part of the diel patterns observed in the same  $c_p$  signal (and inversely), which is probably the case of bio-detritus. We recommend improved  $c_p$  budgets, with simultaneous determinations of the size distribution and refractive index of the individual particles (including heterotrophs and detritus), in addition to their individual concentration.

A quantitative estimation of the respective parts of the numerical concentration, size and refractive index (imaginary and real parts) in the diel variability in  $c_p$  requires simultaneous determinations of all of these quantities. Because the refractive index was not determined concurrently to the particle concentration and size here, it is not possible to reach quantitative conclusions. The refractive index of the particles cannot be measured on a routine basis during field experiments. However, it can be estimated indirectly using various methods based on Mie computations and i) simultaneous measurements of spectral  $c_p$  and backscattering coefficient (Twardowski et al., 2001; backscattering coefficient measurements were unfortunately not made during the present study) or ii) flow cytometry measurements (FCM-Mie method; Green et al., 2003). Such computations are both limited by some underlying assumptions (extensively discussed in the corresponding studies), but are strongly recommended when possible for a complete understanding of the diel patterns in  $c_p$ .

The daily increase in  $c_p$  coefficient (difference between maximum and minimum values) has been used in previous studies to estimate phytoplankton growth rate and daily phytoplankton production (Binder and DuRand, 2002 and references therein). These estimations rely on various assumptions/caveats (see detailed list in Binder and DuRand (2002)) including the assumptions that i) phytoplankton is the sole contributor to diel variations in  $c_p$ , and ii) the conversion factor between  $c_p$  and particulate organic carbon concentration is constant (see introduction). In Ionian Sea waters, the first assumption does not hold, as heterotrophic bacteria appear to play a major role in the diel cycle in  $c_p$ . Community production estimates are still possible from high temporal resolution measurements of  $c_p$  (e.g., Claustre et al.,

2007) if the diel variations in the carbon-specific particle attenuation coefficients  $c^*_c$  are known. Note that to date no studies have been conducted on the  $c^*_c$  of heterotrophs (bacteria, ciliates and flagellates) and its diel patterns. The only available indirect estimation was computed by difference by Claustre et al. (2002). This is definitively required for accurate conversions of the diel variations in  $c_p$  in terms of POC, in particular in oligotrophic systems where the role of heterotrophs appears to be predominant, based on the present study. Ultimately, the improvement of  $c_p$  budgets to compute the contribution of individual stocks (algal, various heterotrophs and detritus) to  $c_p$  (see Oubelkheir et al., 2005) together with the estimation of these budgets with a high temporal frequency would give us access to the production associated with each individual stock, if the corresponding values of  $c^*_c$  (and their associated diel variations) are known.

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