JOURNAL OF PLANKTON RESEARCH | VOLUME 24 | NUMBER 7 | PAGES 635–651 | 2002

# Short-timescale variability of picophytoplankton abundance and cellular parameters in surface waters of the Alboran Sea (western Mediterranean)

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*The Alboran Sea (western Mediterranean) is characterized by a well-defined hydrological structure, the Almeria (Spain)–Oran (Algeria) geostrophic front. During the Almofront-2 cruise (November 22, 1997 to January 18, 1998), high frequency sampling ($\Delta t$ = 0.5 h) of autotrophic picoplankton in surface waters was performed for 17 days (December 24 to January 9) using an automatic sampler. Cell abundance and several cellular parameters were measured by flow cytometry (FCM): forward and right-angle light scatters (FALS, RALS), phycoerythrin (PE) and chlorophyll (Chl) fluorescence. Analysis of abundance of Prochlorococcus, Synechococcus and picoeukaryotes allowed the distinction of two major types of systems: mesotrophic conditions (with waters characterized by detectable nutrients) dominated by picoeukaryotes and more oligotrophic areas (with low to undetectable nutrient levels) dominated by Prochlorococcus and, to a lower extent, Synechococcus. Most of the cellular parameters exhibited a clear diel periodicity, suggesting that cell growth and division processes were tightly coupled to the daily light cycle despite strong gradients of temperature and salinity. Cell growth for both Synechococcus and picoeukaryotes began at dawn and stopped at dusk. In contrast, the increase of Prochlorococcus light scattering only began in late afternoon, a very unusual behaviour that was apparently associated with strong quenching of Prochlorococcus Chl fluorescence during the day. Our data also suggested Chl quenching in Synechococcus but not in picoeukaryotes. Fourier analysis established unambiguously the 24 h diel periodicity for all cellular parameters as well as for cryptophyte abundance, but not for the abundance of other phytoplankters. The burst of division for picophytoplanktonic cells, inferred from the timing of the decrease of light scatter, occurred at dusk for both Synechococcus and picoeukaryotes, and later at night for Prochlorococcus. The ratio between maximum and minimum scatter ($RALS_{max/min}$) was useful in assessing the physiological state of the different picoplankters: high values suggested that populations probably had high division rates, especially picoeukaryotes, suggesting a quasi-optimal physiological activity of the cells in all water types encountered during this cruise despite strong hydrological gradients. This study reveals that the growth rate of the populations may be very little in these ecosystems.*

## INTRODUCTION

Picoplankton variability has been little studied to date [e.g. (Weisse, 1993; Vaulot and Marie, 1999)] and most studies have considered long-term patterns (Campbell *et al.*, 1997; Li, 1998). Until recently, most oceanographic time series have indeed used the month, the week or, in the most extreme case, the day as their minimum sampling period. However, short timescales, in particular the daily scale, are arguably more relevant to understanding the physiology and ecology of these microbial communities. Cells divide very rapidly (up to once per day or more) and respond

very rapidly to environmental fluctuations, such as variation in cloud cover (Jacquet *et al.*, 1998a), vertical mixing (Vaulot and Marie, 1999), or nutrient pulses (Vaulot *et al.*, 1996). Thus, exploring picoplankton population variability on short timescales is critical to obtaining a better understanding of the factors that limit and regulate these cells, i.e. the factors that structure their growth and loss processes. It is of particular interest since small changes in these short-term processes are likely to drive long-term changes in cell populations (Vaulot and Marie, 1999).

The natural alternation of day and night is one of the major events used to interpret short-timescale patterns of picoplanktonic populations. Diel variations in cell abundance, growth and division have been well documented for these cells in the field (Waterbury *et al.*, 1986; Vaulot and Marie, 1999). Whether these diel variations may simply be explained by a direct control of light (Spudich and Sager, 1980) or by a circadian clock (Sweeney and Borgese, 1989) is still unclear. Recently, cell division in *Synechococcus* spp. has been demonstrated to obey a clock-controlled circadian regulation [(Ishiura *et al.*, 1998; Johnson and Golden, 1999) and references therein]. Other important processes for picoplankton dynamics include grazing pressure (Dolan and Simek, 1999), viral attack (Cottrell and Suttle, 1995), mixing and turbulence (Lindell and Post, 1995) and nutrient availability (Vaulot *et al.*, 1996).

Data on short-timescale variability of picoplankton have almost all been obtained in locations with little influence from physical forcings (Vaulot and Marie, 1999). The Almeria–Oran frontal structure of the Alboran Sea (Tintore *et al.*, 1988), investigated during the Almofront-2 cruise (November 22, 1997 to January 18, 1998), offered the possibility to study picoplankton variability in an area of strong hydrological gradients. The use of an automatic water sampler allowed us to collect surface waters at high frequency (every 30 min) and to examine the horizontal distribution and variability of picophytoplankton populations for 17 consecutive days. The goal of this study was to address the following questions. Firstly, is it still possible to observe periodic (in particular diel) patterns in such a dynamic environment? Secondly, what coupling can be observed between cellular parameters and environmental variables on a variety of temporal and spatial scales? Thirdly, is the growth rate affected by strong hydrological gradients?

## METHOD

### Sample collection

The Almofront-2 cruise took place in the Alboran Sea (western Mediterranean Sea) between longitude 0° and 3°W and between latitude 35° and 38°N (Figure 1). Sampling of surface waters was performed for 17 consecutive days during leg 2 of the cruise, i.e. between December 24, 1997 and January 9, 1998. Overall, 750 samples were collected. Leg 2 of the cruise was devoted to documenting physical, chemical and biological characteristics of the Alboran Sea at selected sites. The need to locate these sites precisely (enumerated from 1 to 7) across the jet-front-gyre system as symbolized on Figure 2 explains the tangled vessel route (Figure 1). Surface seawater samples were obtained using a compact automatic sampler [modified from (Jacquet *et al.*, 1998b); see also details at http://www.sb-roscoff.fr/Phyto/SJ_sampler_98.html]. The sampler was connected to the output of an onboard thermosalinograph (SBE 21, Seabird, Seattle, USA) that delivered water continuously sampled from a depth of 4 m under the vessel hull and monitored salinity and temperature every 6 min. From the thermosalinograph output, a peristaltic pump (Masterflex® L/S™, Bioblock, Illkirch, Belgium) retrieved water through 6 m long tubing (Tygon®, 6419–14, Masterflex) and was programmed to deliver about 3 ml every 30 min to tubes kept at 4°C. The tubes, filled with surface sea water, were maintained in these conditions for a maximum delay of 6 h before sample fixation. It was previously shown that this protocol results in minimal effects on parameters such as abundance, size and pigment fluorescence of picoplanktonic cells (Jacquet *et al.*,

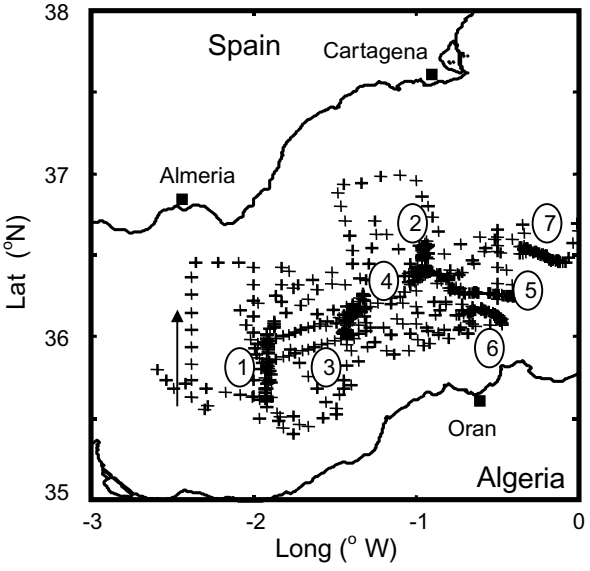


**Fig. 1.** The Alboran Sea with 30 min interval sampling locations during this study symbolized by +. Sites correspond to well-defined hydrological situations, i.e. 'Mediterranean waters' (site 2), the Atlantic baroclinic jet (sites 1, 4, 5), the Almeria-Oran front (site 7) and the eastern Alboran gyre (sites 3, 6). The arrow indicates the beginning and the direction of sampling.

1998b). Briefly, there were no significant differences in the abundance, chlorophyll (Chl) and phycoerythrin (PE) fluorescence and cell cycle data of *Prochlorococcus* and *Synechococcus* between populations analysed directly after sampling or after 10 h of 4°C preservation. The only ratio (preserved : control) different from 1.0 was that for *Prochlorococcus* right-angle light scatter [RALS; 0.93, (Jacquet *et al.*, 1998b)]. Before each sampling, the 6 m long tubing between the thermo-salinograph and the sampler was automatically rinsed for 4 min.

## Sample processing and flow cytometric data analysis

Samples were fixed with a mixture of glutaraldehyde and paraformaldehyde [0.05 and 1% final concentration, respectively; see (Jacquet *et al.*, 1998a)] for 15 min in subdued light conditions and then frozen in liquid nitrogen for delayed analysis. Prior to analysis, samples were rapidly thawed at 37°C. Samples were analysed with a FACSort™ flow cytometer (FCM, Becton Dickinson, San Jose, CA) as previously described (Marie *et al.*, 1999). Use of the FCM provided autotrophic population counts, the forward and right-angle light scatters (FALS and RALS) and pigment characteristics (Chl and PE content) of the cells. *Prochlorococcus* cells were easily discriminated from background noise despite their dim Chl fluorescence (not shown). Cryptophytes, displaying orange fluorescence, were discriminated from *Synechococcus* because of their greater red chlorophyll fluorescence (not shown). We verified using epifluorescence microscopy (EFM) following FCM sorting that the larger (in terms of cell size) population was indeed related to cryptophytes and not to large cyanobacteria as reported in the equatorial Pacific Ocean by Neveux *et al.* (Neveux *et al.*, 1999). This could be easily inferred from the observation of a well-defined chloroplast inside the cells. Fluorescent beads (0.95 μm diameter; Polyscience, Warrington, USA) were added to each sample and all the parameters were normalized to them. FCM listmode files were analysed using the custom-designed freeware CYTOWIN [modified from (Vaulot, 1989); available at http://www.sb-roscoff.fr/Phyto/cyto.html]. A Fourier analysis was applied to the data to extract, if present, frequency peaks relating some periodic patterns. For this, the ORIGIN 4.1 software package (Microcal Software) was used.

## Environmental data sets

Environmental factors, i.e. salinity (g $l^{-1}$), temperature (°C), natural irradiance (W $m^{-2}$) and wind force (measured in knots) were recorded in real time on board (Figure 3). Measurements of inorganic nutrient concentrations in surface waters (Table I) were carried out using a Technicon AutoAnalyser II (precision for nutrient measurements was 0.01 μM). Surface current speed was acquired with a 300 kHz vessel-mounted RDI® Acoustic Doppler Current Profiler (not shown).

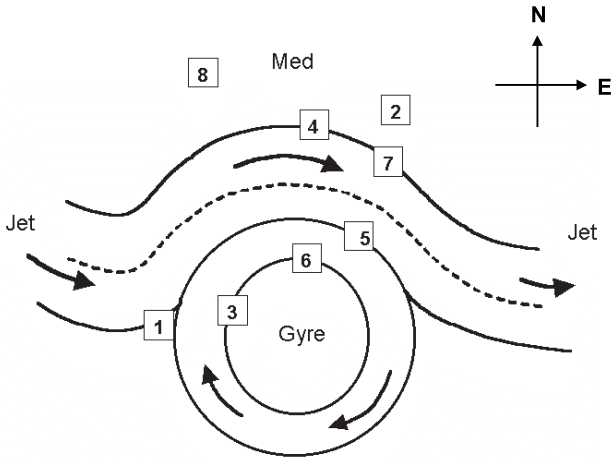


**Fig. 2.** Schematic positions of sites in the jet-front-gyre system explored during leg-2 of the cruise.

# RESULTS

## Environmental conditions

In the following text we will refer to typical waters of the Mediterranean basin and those originating from the Atlantic as 'Mediterranean waters' and '(modified) Atlantic waters', respectively. The two types of water were well identified from both salinity (Figure 3A) and temperature (Figure 3B) measurements; Mediterranean waters being more saline and colder than Atlantic ones. The different sites investigated during leg 2 of the cruise (enumerated from 1 to 7 on Figures 1 and 2) could be easily located from these signatures as indicated on panel 3A. The strong surface gradient of temperature and salinity recorded on January 8 (site 7) corresponded to the Almeria–Oran geostrophic front separating Atlantic and Mediterranean waters [see (Prieur and Sournia, 1994; Claustre *et al.*, 2000) for more details]. Solar irradiance (Figure 3C) was low during the period of study, reaching exceptionally 700 W $m^{-2}$ on December 25. Some days were very cloudy (for example December 26, 1997 or January 1 and 2, 1998) and maximal irradiance recorded on these days remained below 400 W $m^{-2}$. Wind speed (Figure 3D) was generally low but occasionally reached 15 knots (on December 26, 28, 31 and on January 1, 2). Lowest nutrient concentrations were recorded at sites 2 and 7 (Table I). In these Mediterranean and frontal-influenced waters, phosphates were never detected (the limit of detection being 10 nmol $l^{-1}$) and nitrates were below 0.06

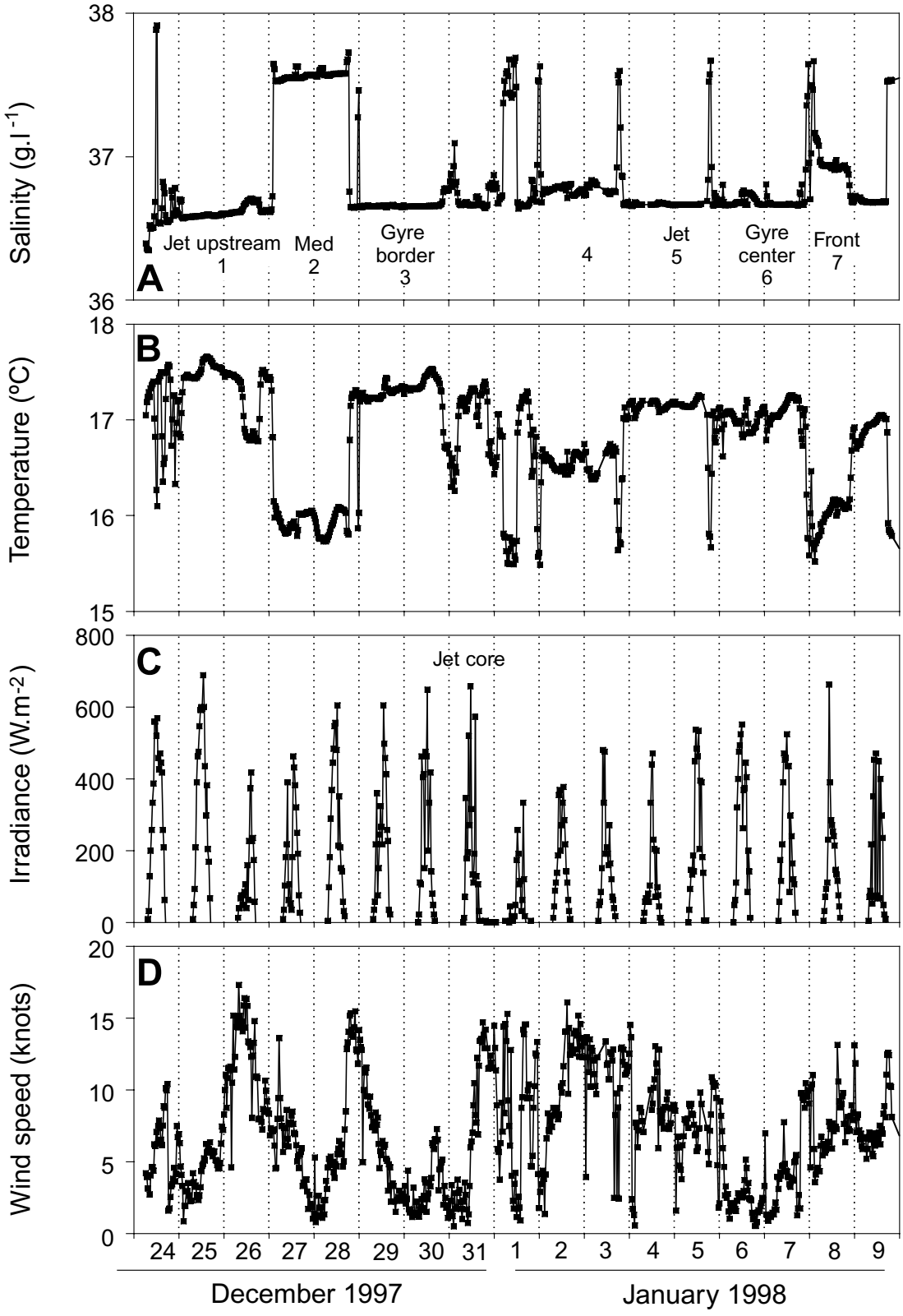


**Fig. 3.** Chemical and physical parameters recorded every 30 min between December 24, 1997 and January 9, 1998: (**A**) salinity (PSU), (**B**) temperature (°C), (**C**) solar irradiance (W $m^{-2}$) and (**D**) wind force (knots). Source NO 'l'Atalante'.

*Table I: Nitrate [N-$NO_3$] and phosphate [P-$PO_4$] concentrations (in μmol $l^{-1}$) in surface waters of the Alboran Sea*

| Site | CTD cast | Depth (m) | [N-$NO_3$] | [P-$PO_4$] |
|---|---|---|---|---|
| 1 | 403 | 3.0 | 0.77 | 0.03 |
| 1 | 406 | 5.3 | 0.80 | 0.04 |
| 1 | 410 | 3.9 | 0.78 | 0.02 |
| 2 | 416 | 6.7 | 0.06 | 0.00 |
| 2 | 419 | 5.0 | 0.06 | 0.00 |
| 2 | 423 | 3.1 | 0.06 | 0.00 |
| 3 | 429 | 4.8 | 0.78 | 0.01 |
| 3 | 432 | 5.3 | 0.74 | 0.04 |
| 3 | 436 | 3.0 | 0.71 | 0.01 |
| 4 | 496 | 3.6 | 0.37 | 0.00 |
| 4 | 499 | 4.6 | 0.43 | 0.01 |
| 4 | 503 | 4.0 | 1.01 | 0.02 |
| 5 | 509 | 2.9 | 0.56 | 0.01 |
| 5 | 512 | 4.2 | 0.51 | 0.01 |
| 5 | 516 | 4.4 | 0.61 | 0.01 |
| 6 | 522 | 5.5 | 0.53 | 0.01 |
| 6 | 526 | 4.8 | 0.43 | 0.02 |
| 6 | 530 | 2.6 | 0.55 | 0.02 |
| 7 | 537 | 3.3 | 0.24 | 0.00 |
| 7 | 540 | 4.9 | 0.12 | 0.00 |
| 7 | 544 | 3.5 | 0.09 | 0.00 |

Site number, CTD cast and real depth of sampling are given for each station. Measurements were obtained using a Technicon Autoanalyzer II. Data courtesy of P. Morin.

(site 2) and 0.24 μmol $l^{-1}$ (site 7), respectively. In contrast, Atlantic waters were not depleted in phosphates and nitrates, the concentrations of which ranged between 0.01 and 0.04 μmol $l^{-1}$ and between 0.37 and 1.01 μmol $l^{-1}$, respectively. It is noteworthy that the full range of variation for nitrates was recorded at a single site, i.e. at site 4 (jet core), where, except for the high value of 1.01 μmol $l^{-1}$, nutrient concentrations were on average lower than at other Atlantic sites. Elsewhere the range of nutrient variations was clearly smaller (Table I).

## Abundance of picophytoplankton

Figures 4 and 5 respectively provide global and detailed views of the distribution of the different groups in the surface waters of the Alboran Sea. Cell abundance of *Prochlorococcus* varied between $2 \times 10^3$ and $65 \times 10^3$ cells $ml^{-1}$, with an average of less than $10 \times 10^3$ cells $ml^{-1}$ (Figures 4A, 5A). Cell concentrations of *Prochlorococcus* were generally higher in Mediterranean waters (i.e. above $20 \times 10^3$ cells $ml^{-1}$) and lower in Atlantic waters ($<10 \times 10^3$ cells $ml^{-1}$). The highest concentrations were recorded on January 1, on the transect between sites 3 and 4, as the ship passed through Mediterranean waters. The Mediterranean waters were clearly influenced by the frontal structure, as revealed by the variations recorded in both temperature and salinity at this place (Figures 3A, B). *Synechococcus* were also well represented in Mediterranean waters, but Atlantic and Mediterranean sea waters differed much less than for *Prochlorococcus*. The average cell concentration of *Synechococcus* was exactly twice that of

*Prochlorococcus*, i.e. about $16 \times 10^3$ cells ml$^{-1}$ (Figures 4B, 5B). Clear maxima of *Synechococcus* cell numbers were observed on December 30 and 31 and on January 8 and 9 near the frontal structure, and concentrations reached about $30 \times 10^3$ and $45 \times 10^3$ cells ml$^{-1}$, respectively. At site 7, the highest values were clearly recorded inside the frontal structure and in both Mediterranean and Atlantic adjacent waters (Figure 5B). On January 1, increased concentration was recorded as for *Prochlorococcus*, but to a lower extent. For both *Prochlorococcus* and *Synechococcus*, it was not possible to infer, based on FCM cytograms, whether the populations of Mediterranean waters and of Atlantic jet waters were either phenotypically and/or genetically similar or different. In contrast to prokaryotes, both picoeukaryotes and cryptophytes were poorly represented in Mediterranean waters and dominated in modified Atlantic waters. Picoeukaryotes were on average (with $11 \times 10^3$ cells ml$^{-1}$) less abundant than *Synechococcus*, but more than *Prochlorococcus (*Figures 4C, 5C). Their concentration varied more than 10-fold between $2 \times 10^3$ and $35 \times 10^3$ cells ml$^{-1}$. As observed for *Synechococcus*, a clear enhancement of cell concentration of this community was recorded between December 30 and 31 (Figure 5C), but not in the front at site 7. Other elevated concentrations were recorded at site 1, i.e. in Atlantic waters inside the jet. Cryptophyte cell numbers rarely exceeded 100 cells ml$^{-1}$ (Figures 4D, 5D). The average cell concentration was only 60 cells ml$^{-1}$. Maximum cell numbers were essentially recorded in modified Atlantic waters. This population was the sole for which a Fourier analysis (not shown) revealed a clear diel periodicity in cell abundance with maxima and minima generally recorded during daylight and at early night, respectively (Figure 5D).

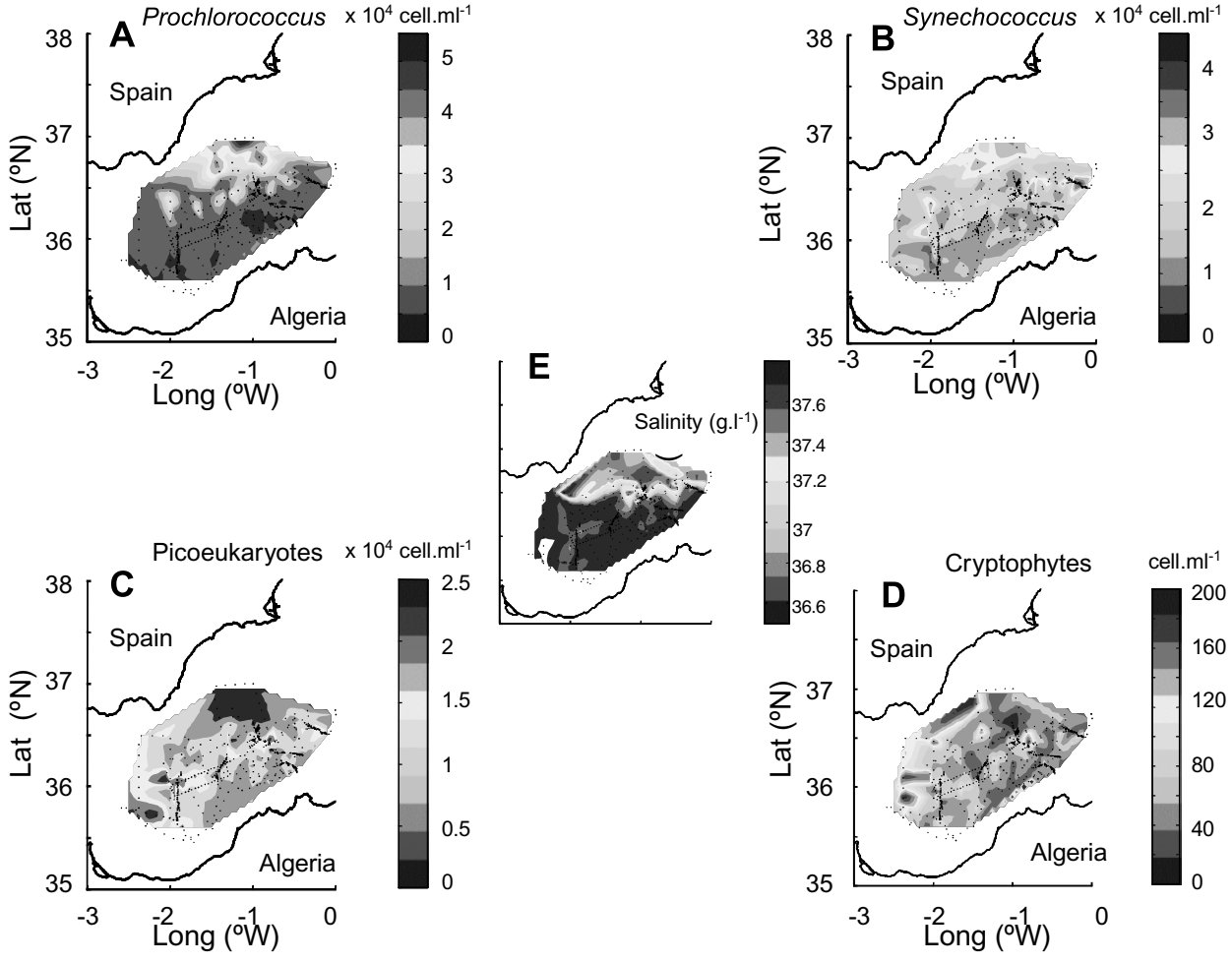


**Fig. 4.** Maps of cell abundance for *Prochlorococcus* (**A**), *Synechococcus* (**B**), picoeukaryotes (**C**), cryptophytes (**D**) and of salinity (**E**) in surface waters of the Alboran Sea between December 24, 1997 and January 9, 1998.

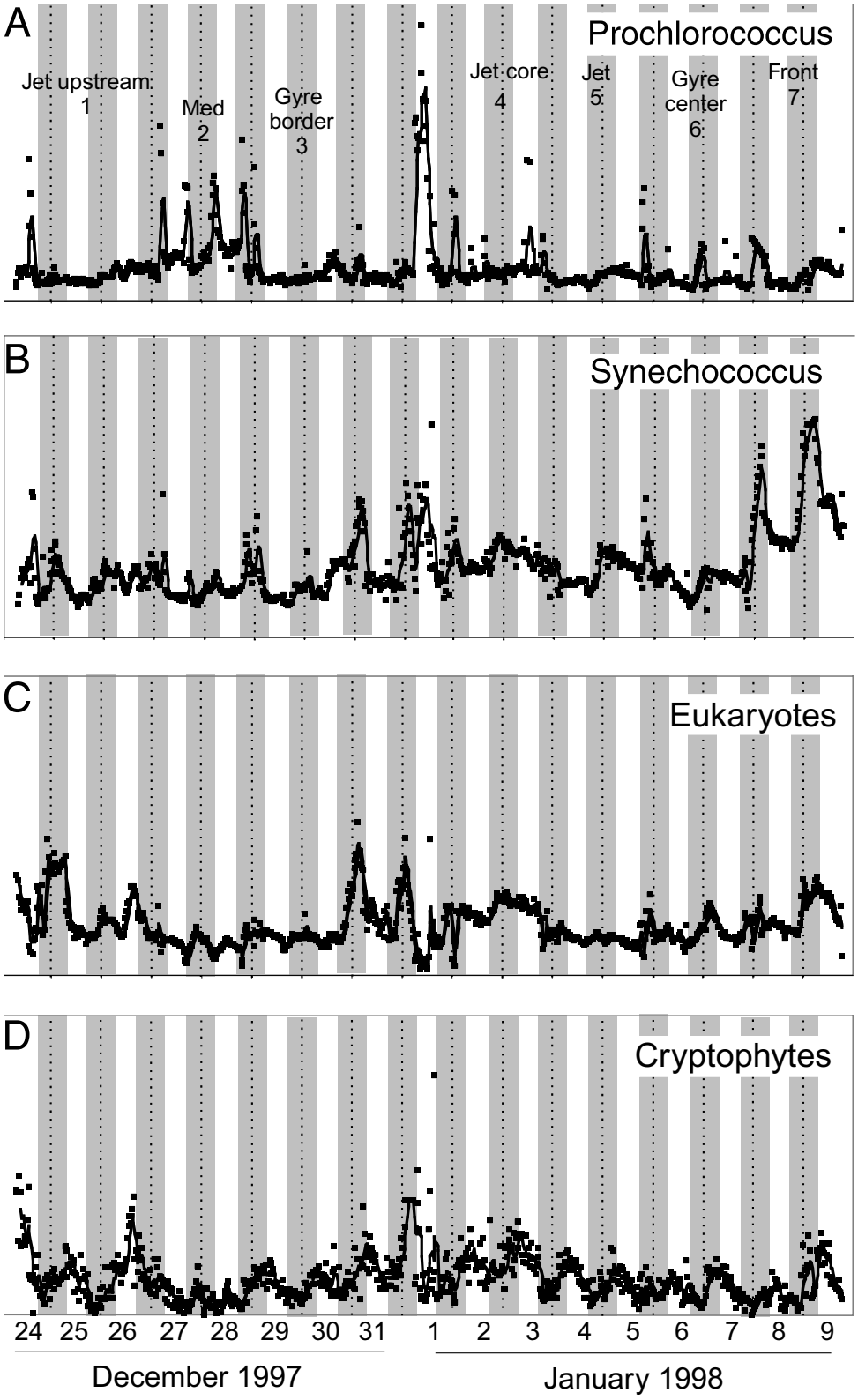


**Fig. 5.** Cell abundance of *Prochlorococcus* (**A**), *Synechococcus* (**B**), picoeukaryotes (**C**) and cryptophytes (**D**) in surface waters of the Alboran Sea sampled every 30 min between December 24, 1997 and January 9, 1998. Curves fitting the data correspond to a third-order moving average. Vertical grey bars symbolize periods of darkness.

## Light scatter and pigment fluorescence of picophytoplankton

Both light scattering and Chl fluorescence of the three autotrophic picoplanktonic groups (*Prochlorococcus*, *Synechococcus* and picoeukaryotes) displayed some diel patterns (Figures 6, 8) that were unambiguously confirmed by Fourier analysis. Cryptophytes were not abundant enough to allow reliable analysis of cellular parameters. We chose to represent FALS for *Synechococcus* (Figure 6C) since it displayed clearer patterns than RALS. It is noteworthy that FALS, i.e. light scatter at low angles relative to the excitation light, is generally accepted as a proxy of cell size that increases linearly with the square of the cell diameter or cross-section (Cunninghan and Buonaccorsi, 1992). By comparison, the RALS reflects particle morphology and structure more (Dubelaar *et al.*, 1987; Ackelson *et al.*, 1988). However, for picophytoplankton, and especially for the very small *Prochlorococcus*, RALS often provides a better measure of relative cell size because of higher sensitivity to small changes as compared to FALS. Since the FALS signal is much more noisy than RALS (and therefore generates more scatter in the data), we preferred to show RALS for the other groups. Patterns for light scattering observed for *Synechococcus* and picoeukaryotes were very similar, with an increase beginning at the onset of light and maximal values recorded around dusk (Figures 6C,D). By contrast, the increase of *Prochlorococcus* RALS (or FALS) began a few hours before the light to dark transition (Figures 6B, 9C,D) except on January 1; this is an unusual pattern compared to previous reports (see Discussion). The RALS decrease was recorded about 4–6 h later than that of *Synechococcus* and picoeukaryotes. Assuming that the beginning of the scatter decrease was linked to the burst of division, *Synechococcus* and picoeukaryotes were observed to divide at approximately the same time, i.e. between 17:00 and 18:00 h local time corresponding to sunset. *Prochlorococcus* divided later at night, around midnight (Figure 6B). As said above, cryptophytes also seemed to divide at night. The daily averaged value for RALS of each group varied only slightly over the area of study (Figure 7). The lowest mean values for *Prochlorococcus* RALS were associated with typical or frontal-influenced Mediterranean waters (Figure 7A), but no obvious trend was observed for either *Synechococcus* or picoeukaryotes (Figure 7B,C) in terms of water-type influence. For all groups, the lowest mean value was clearly recorded on January 8 or 9 in association with the front (site 7). The amplitude of RALS variation (given by the $RALS_{max/min}$ ratio) changed also from day to day and with populations. A higher range of variation for this ratio was recorded for picoeukaryotes (1.4–2.8), then for *Prochlorococcus* (1.3–1.9) followed by *Synechococcus* (1.2–1.4). *Prochlorococcus* was the only one for which we found a weak correlation between $RALS_{max/min}$ ratio and daily integrated irradiance ($r^2$ = 0.6, $n$ = 17, not shown) throughout the period of study (Figure 7D). The three picoplankters displayed a low $RALS_{max/min}$ ratio, especially for picoeukaryotes on December 26 (Figure 7F). Interestingly, this corresponded to the lowest daily integrated irradiance as well as the windiest day. However, no clear linear correlations were observed between $RALS_{max/min}$ and the integrated irradiance each day for *Synechococcus* and picoeukaryotes, suggesting that other factors were involved. Typically, very low values for $RALS_{max/min}$ were also recorded on January 2, 3 and 4, corresponding to low irradiance, particularly windy days and low nutrient concentrations, and on January 8 and 9, corresponding to frontal waters (see Discussion).

Chlorophyll fluorescence patterns during the light period displayed differences between groups. In contrast, at night, all cell populations exhibited a similar behaviour with a net decrease in fluorescence after cell division (Figure 8). *Prochlorococcus* was characterized by a 'three-step' variation in Chl fluorescence on the sunniest days with a decrease of fluorescence recorded during the first part of the day, followed by an increase during the second part of the day and finally a decrease at night (Figure 8B). This clear decrease recorded for *Prochlorococcus* fluorescence during the day was probably associated with non-photochemical quenching due to the high irradiance experienced by the cells in surface waters. Clear variations in the timing of maximum fluorescence quenching could be recorded from day to day in response to daily irradiance fluctuations. As an example, quenching recorded for *Prochlorococcus* on December 27 was delayed compared to that on January 6 (Figures 9E,F) and this variation could be directly associated with marked variations of irradiance level around midday (Figures 9A,C). Fluorescence quenching was not recorded on the cloudiest day, i.e. on January 1 (Figure 8B). For *Synechococcus*, the patterns obtained for cellular chlorophyll were noisy (Figure 8B), and only Fourier analysis clearly revealed the diel periodicity. The clearest diel pattern was recorded on January 1, which corresponded to the cloudiest day. Although it was difficult to determine whether these cells experienced photo-quenching on sunniest days, a weak decrease of *Synechococcus* Chl fluorescence was observed around midday on most occasions (see for example December 27 or January 9). On December 27 and 28, very low variation in Chl fluorescence was recorded compared to the days before and after. It is likely that a shift from Mediterranean to Atlantic waters induced this step up of the Chl fluorescence values (Figure 10). Indeed, irradiance level did not change significantly and therefore could not explain such variations. The tight PE vs. Chl fluorescence correlation

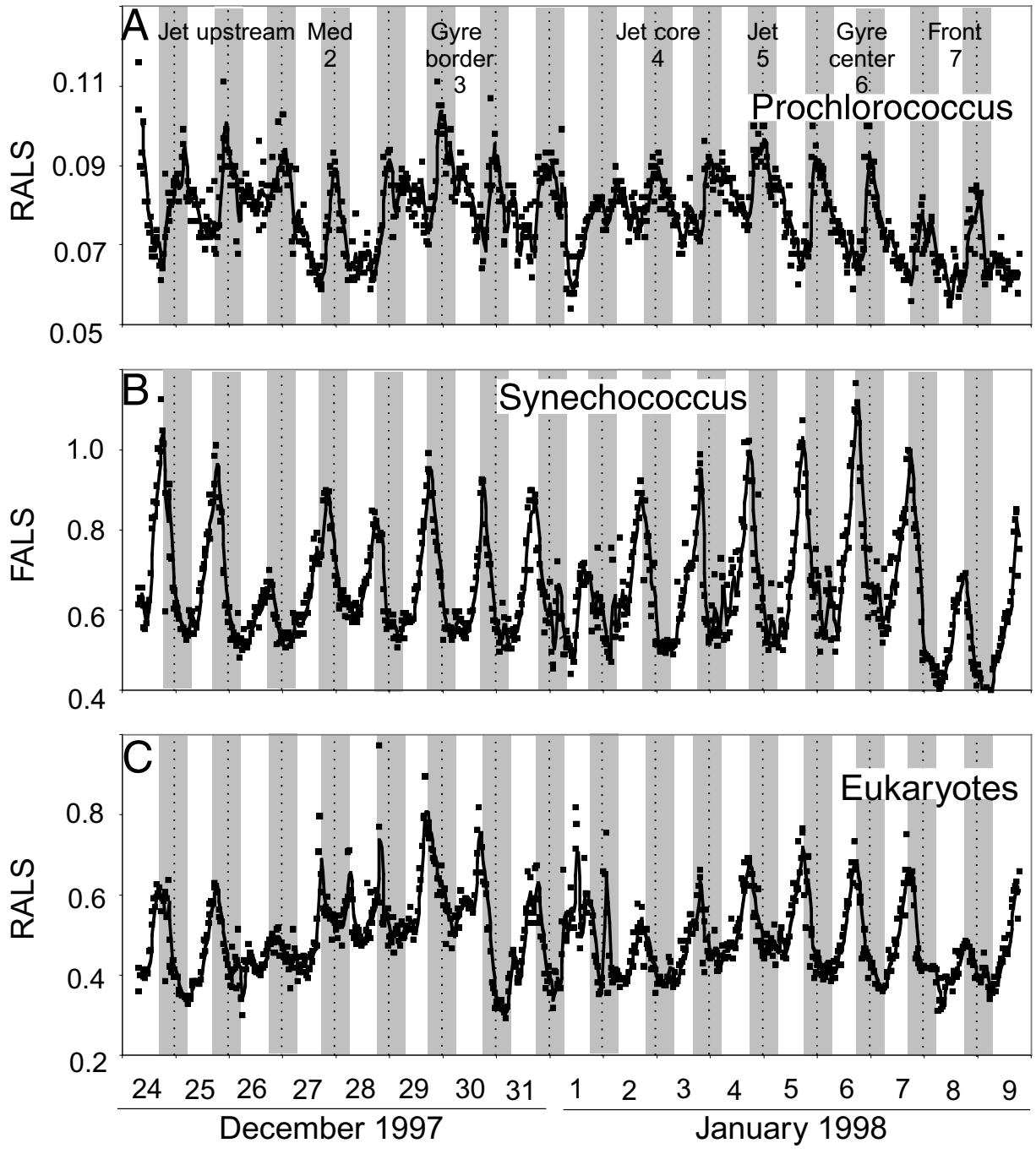


**Fig. 6.** Light scatter (RALS or FALS) of *Prochlorococcus* (**A**), *Synechococcus* (**B**) and picoeukaryotes (**C**) sampled every 30 min between December 24, 1997 and January 9, 1998. Curves and symbols as on Figure 5.

(PE = 0.31[Chl] – 0.13, $r^2 = 0.95$, $P < 0.001$, $n = 715$, not shown) precludes a dramatic shift in population (e.g. from PE- to PC-containing cyanobacteria), although more subtle changes in genetic variation may have occurred. However, the variation was most probably due to the increase in nutrients associated with Atlantic waters (see Discussion). As for light scatter, a narrow diel variation in Chl fluorescence of *Synechococcus* was also recorded on January 8 (i.e. in frontal waters) in addition to very low relative values. The clearest daily pattern recorded for Chl fluorescence was that for picoeukaryotes. No clear photoquenching was recorded for this community (Figure 8D).

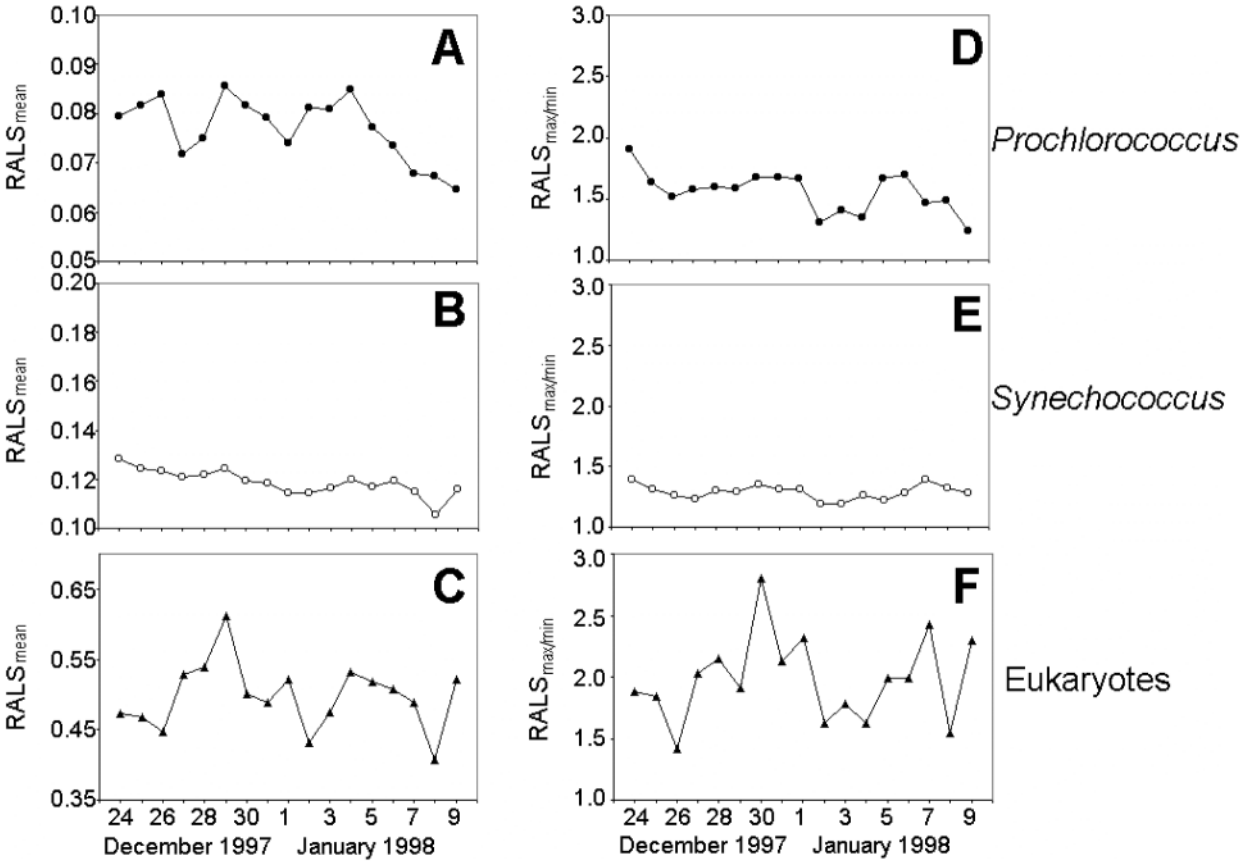


**Fig. 7.** Daily mean value of RALS and of the $RALS_{max/min}$ ratio for *Prochlorococcus* (**A**, **D**), *Synechococcus* (**B**, **E**) and picoeukaryotic cells (**C**, **F**) between December 24, 1997 and January 9, 1998.

As in the case of *Synechococcus*, some low relative values and variations for chlorophyll fluorescence were also recorded on December 27 and 28 (site 2). In addition, lowest relative values and range of variations were recorded on January 8, corresponding to frontal waters, as for the other groups.

## DISCUSSION

Little is known about the impact of environmental factors on the short-term dynamics of phytoplankton populations. Small-scale variability for picoplankton abundance and cellular parameters has been studied on several occasions in the open ocean and has revealed important daily variations generated by temporary imbalances between growth and loss processes (Binder *et al.*, 1996; Vaulot and Marie, 1999). Mesoscale influence strongly modifies the necessary conditions for primary production, i.e. nutrient and light availability (Levy *et al.*, 2000) and variations recorded in surface waters are likely to be representative of processes throughout the euphotic zone (Partensky *et al.*, 1999; Tarran *et al.*, 1999). In this context, the surface waters of the Alboran Sea are of interest since it is a region with strong horizontal and vertical forcings. In the following discussion, we examine first the distribution of populations in response to environmental factors and second how these factors may influence the patterns recorded for their cellular parameters and growth rates.

### Distribution of cell abundance

Analysis of the abundance of *Prochlorococcus*, *Synechococcus* and picoeukaryotes allowed the distinction of two major types of systems: mesotrophic conditions (Atlantic waters, detectable nutrient levels) dominated by eukaryotes (including cryptophytes) and *Synechococcus*, and more oligotrophic areas (Mediterranean sea waters, low to undetectable nutrient levels) dominated by *Prochlorococcus* and, to a less extent, *Synechococcus*. The contribution of picophytoplankton in terms of chl *a* to the total <200 μm fraction reached almost 25% and 50% in these oligotrophic and mesotrophic areas, respectively and it was essentially

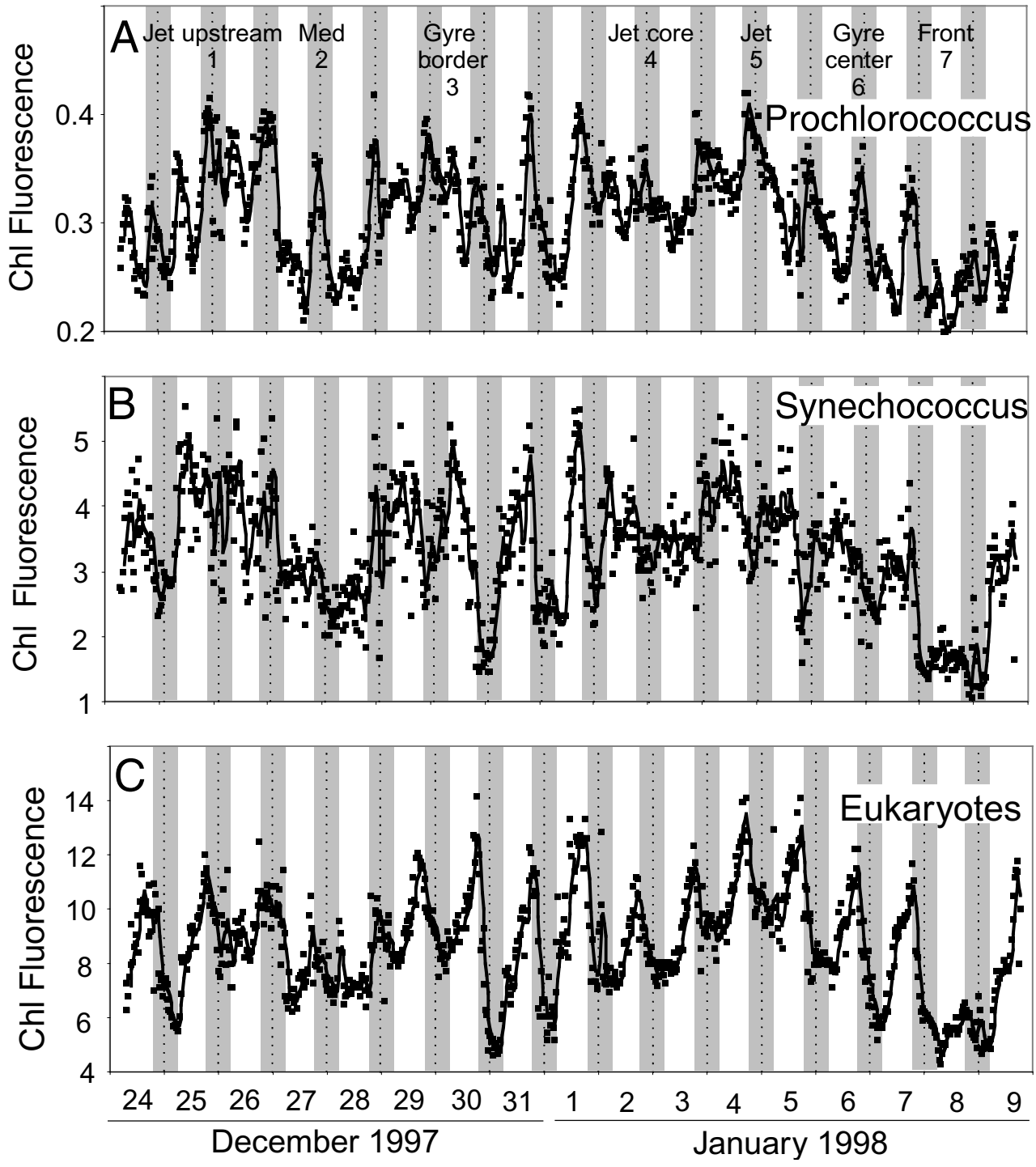


**Fig. 8.** Red chlorophyll (Chl) fluorescence of *Prochlorococcus* (**A**), *Synechococcus* (**B**) and picoeukaryotes (**C**) sampled every 30 min between December 24, 1997 and January 9, 1998. Curves and symbols as on Figure 5.

due to picoeukaryotes in the latter case (Jacquet *et al.*, unpublished data). Such a distribution, if only related to nutrient level, is in agreement with a wide range of previous observations (Partensky *et al.*, 1996; Zubkov *et al.*, 1998). *Prochlorococcus* exhibited on average the lowest concentration of the three picophytoplankters; *Synechococcus* abundance was twice that of *Prochlorococcus* and was slightly higher than that of picoeukaryotes. These ranges

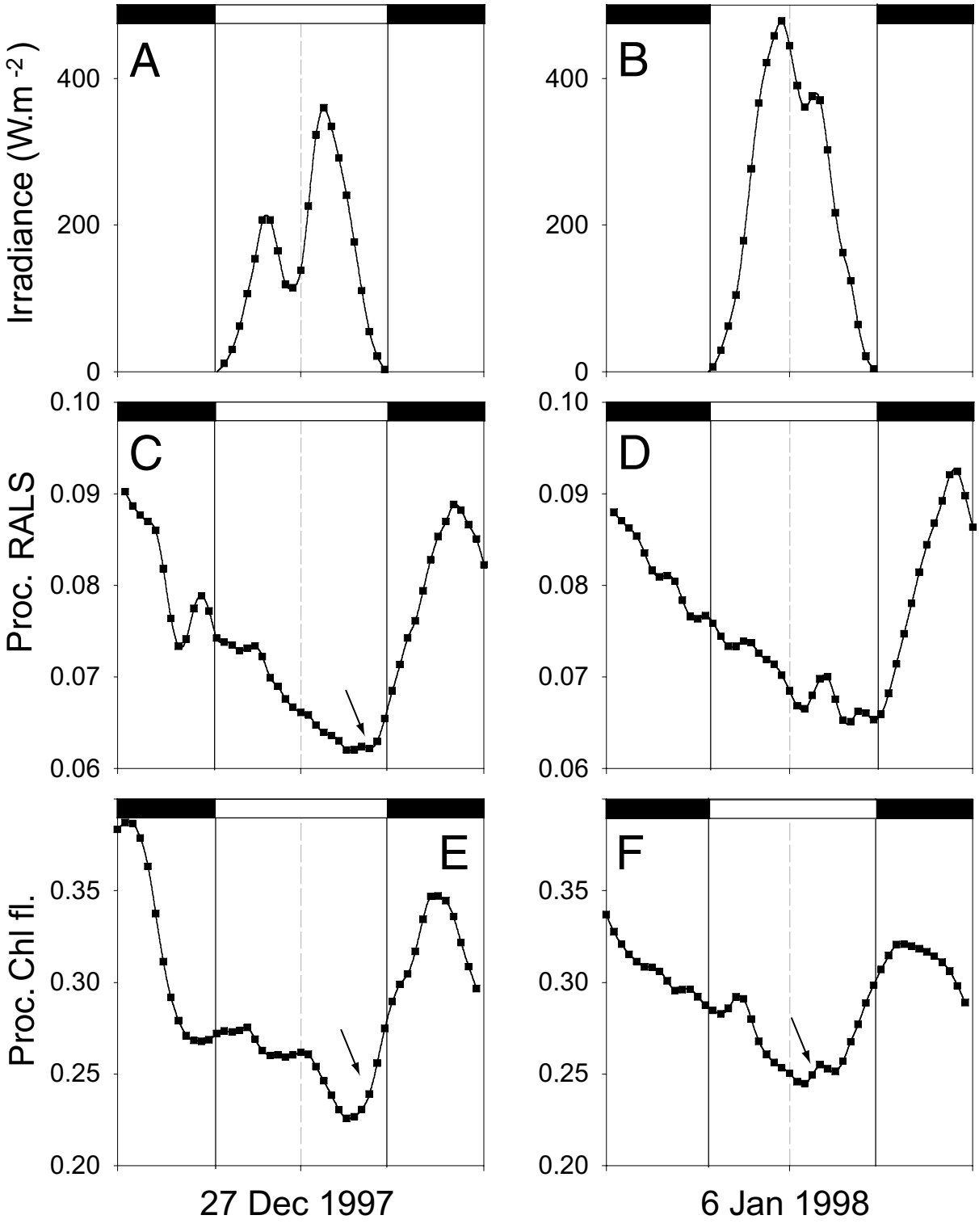


**Fig. 9.** Comparison between two selected days (December 27, 1997 and January 6, 1998) for light irradiance (**A**, **B**), *Prochlorococcus* RALS (**C**, **D**) and Chl fluorescence (E, F). All data were smoothed using a low-pass filter removing frequencies above 0.5 $h^{-1}$ with the equation: $y_i = a_6.x_{i-6} + \ldots + a_0.x_i + \ldots + a_6.x_{i+6}$ with $a_0 = 0.303$, $a_1 = 0.217$, $a_2 = 0.055$, $a_3 = 0.033$, $a_4 = 0.025$, $a_5 = 0.013$, $a_6 = 0.005$, and where $x_i$ and $y_i$ are the original and smoothed data, respectively, at time $t_i$.

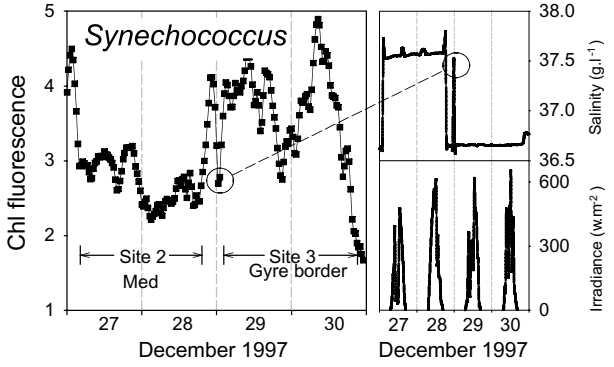


**Fig. 10.** Pattern recorded for Chl fluorescence of *Synechococcus* cells (smoothed data obtained as on Figure 9) between December 27 and 30, 1997 in association with salinity and irradiance fluctuations. The two circles are drawn to symbolize the strong correlation between hydrological conditions and chlorophyll fluorescence of the cells.

of abundance were in agreement with a previous study in the same region in early March 1992 (Vaulot and Marie, 1993) suggesting little year-to-year variation, as observed in more stable ecosystems, such as those of the central oceanic gyres (Campbell *et al.*, 1997). Such an invariance across vast oceanic regions and over a large (annual) temporal scale clearly indicates that growth and loss processes are balanced over these large scales and our data with other recent studies [e.g. (Jacquet *et al.*, 1998a; Vaulot and Marie, 1999)] clearly suggest much larger variations at the daily scale.

High-frequency sample acquisition allowed us to observe clear peaks of high abundance superimposed on a low population background, especially for *Prochlorococcus* (Figure 5). These peaks of abundance were clearly associated with waters of high salinity and low temperature of Mediterranean origin, as for example on January 1. In this case, the influence of the frontal structure (as inferred from gradients of temperature, salinity and current speed) probably induced injection (or favoured horizontal spreading) of nutrients into the surface layer. Moreover the absence of wind on that day (January 1) probably stabilized the water column and allowed *Prochlorococcus*, an organism that clearly favours stratified over well-mixed situations (Lindell and Post, 1995), to respond to elevated nutrient levels; this was previously observed in winter in the western Mediterranean Sea (Vaulot and Partensky, 1992). A higher abundance integrated over the 0–100 m layer of *Prochlorococcus* in frontal waters on the Mediterranean side compared to adjacent Mediterranean and modified Atlantic waters agreed with such a hypothesis (Jacquet *et al.*, unpublished data). As it has been recently suggested that *Prochlorococcus* may not utilize nitrates (Partensky *et al.*, 1999; López-Lozano *et al.*, in press), it is likely that high abundance of the prokaryote at this site was due to the stability of the water column and to the presence of P and another N source such as ammonium and/or urea (Lindell and Post, 2001; Palinska *et al.*, 2000). High concentrations of *Synechococcus* and picoeukaryotes were clearly recorded between December 30 and 31, and of *Synechococcus* alone between January 8 and 9. These samples were taken in high nutrient Atlantic waters close to or within frontal waters during sunny and windy days. In this case, advection induced by strong wind stress could be a key factor in the increased nutrient levels observed (Klein and Coste, 1984). Moreover, strong advection in the frontal zone probably maintained a high level of nitrates in surface waters (Levy *et al.*, 2000). Additionally, under these turbulent conditions, *Synechococcus* and picoeukaryotes were probably favoured over *Prochlorococcus* (Lindell and Post, 1995). Cryptophytes were negatively correlated with salinity and positively correlated with nutrients, i.e. more abundant in modified Atlantic waters. This result is in agreement with what we found during a recent mesocosm experiment demonstrating that high nutrient concentrations are indeed a prerequisite for the growth of these larger cells (Jacquet *et al.*, 2002). It is, however, noteworthy that cryptophytes may be in some cases not counted by FCM in low nutrient conditions as their PE can break down (and therefore not be visible). Additionally, cell abundances of ~10 $ml^{-1}$ strongly suggest that a larger sample volume should be analysed to obtain more reliable data. Our data do not permit us to tell if we may have measured enough cells to test our assumption of the relationship between cryptophytes and available nutrients, and thus it must be considered with caution until more evidence is provided. Except for local peaks in cell numbers, picoplankton population abundance remained remarkably constant. This suggests that growth and loss processes are balanced over a wide range of scales and that spatial changes of hydrological parameters in surface waters may be of limited importance compared to nutrient levels to explain picoplankton population distribution (Zubkov *et al.*, 2000).

## Patterns of picoplankton cellular parameters

The strongest diel periodicity was recorded for the measures of light scattering (both FALS and RALS). For *Synechococcus* and picoeukaryotes, these parameters increased during the light period and decreased at night, consistent with the accumulation of carbon through photosynthesis followed by cell division, producing smaller cells that scatter less (Stramski *et al.*, 1995; DuRand and Olson, 1998). For these two groups of organisms, the increase of intracellular carbon began at dawn, while for *Prochlorococcus*, in most cases, growth occurred only at the end of the daily light period and

stopped later at night. This is clearly unusual since in previous studies, both in the field (Blanchot *et al.*, 1997; Vaulot and Marie, 1999) and in culture (Jacquet *et al.*, 2001a, b), *Prochlorococcus* FALS or RALS increased from the onset of the light period until early in the night. During the Almofront-2 cruise, this important delay in the scattering increase was apparently associated with fluorescence quenching: on January 1, i.e. the day with the lowest irradiance over the period of study, no quenching was recorded and RALS began to increase immediately at sunrise. However, in studies mentioned above [e.g. (Vaulot and Marie, 1999) in the equatorial Pacific], strong quenching was recorded in surface waters without the occurrence of a delay in the increase of light scatter. A possible explanation is that winter Mediterranean populations of *Prochlorococcus* could be much more sensitive to high visible/UV light intensities than those of permanently stratified waters such as those of the equatorial Pacific. Additionally, it is not impossible that strong mixing and turbulence were responsible for disruption of the cell cycle of *Prochlorococcus*, and thus of its diel patterns. Additionally, the effects of a diurnally mixing and stratifying euphotic zone may be considered as a physical process which could disturb the delicate balance between growth and loss processes (Vaulot and Marie, 1999). Indeed, our data reveal that sea-surface temperature varied by about 0.2–0.4°C over the diel cycle. During the period of this study (i.e. early winter), it is likely that the Alboran Sea was, in general, weakly stratified, implying that the large diel sea-surface temperature change may lead to an appreciable deepening of the mixed layer at night. If so, the physiological makeup of mixed-layer algal populations is likely to change at night since low-light acclimated populations from below the mixed layer are advected into the mixed layer. This process may account for the unusual observation that *Prochlorococcus* RALS increases at night. It may well be that at night larger *Prochlorococcus*, growing and residing below the mixed layer during the day, are mixed together with smaller mixed-layer cells, thus increasing population RALS at a time when one would expect RALS to decrease due to cell division and respiration. Such a hypothesis would explain why, on January 1, a day when wind was almost absent and change in sea-surface temperature was weak, the diel variation for *Prochlorococcus* RALS was as expected compared to previous studies (i.e. an increase during the day and a decrease at night). Following this hypothesis, some caution should be exercised before extrapolating scatter variations to division rates since sampling surface waters may not always correspond to the same population, physiologically and genetically speaking (see below).

An attempt was made to analyse the scatter data further. The ratio of the maximum to the minimum light scatter (FALS or RALS) over 1 day has been used as a proxy of *in situ* division rate (Binder *et al.*, 1996; Vaulot and Marie, 1999), although Jacquet *et al.* reported a negative correlation between $FALS_{dusk/dawn}$ and the division rate for *Synechococcus* for Mediterranean surface waters in summer (Jacquet *et al.*, 1998a). In Alboran Sea surface waters, the calculation of this ratio suggested that the growth rate of picoeukaryotes was probably higher than those of *Prochlorococcus* and *Synechococcus*. Indeed, the *Synechococcus* $RALS_{max/min}$ ratio varied only weakly, between 1.2 and 1.4, compared to other groups. Such low variability with similar values (i.e. 1.17–1.37) was recorded by Vaulot and Marie in the surface waters of the equatorial Pacific (5°S, 150°W) in November 1994 (Vaulot and Marie, 1999). For *Prochlorococcus*, we found larger variations than Vaulot and Marie (Vaulot and Marie, 1999) although the absolute values were smaller (i.e. 1.4–1.9 vs. 1.9–2.0). These data would suggest that division rates of *Synechococcus* were very similar in the two areas, whereas *Prochlorococcus* showed lower division rates in the Alboran Sea. Since Vaulot and Marie reported that a $RALS_{max/min} = 2$ corresponded to one division per day for *Prochlorococcus* in the equatorial Pacific (Vaulot and Marie, 1999), the Mediterranean population was likely to divide less than once per day. Of course, this interpretation has to be considered with caution with regards to the discussion above about variations of scatter in *Prochlorococcus*. With this caveat in mind, it is however particularly intriguing to note that our data strongly suggest that the growth rate of these populations may be poorly affected in these ecosystems compared to more stable systems. Thus, it could mean that hydrology is not a key factor in the regulation of the growth rate of these populations. Clearly, light and nutrients appear as the first candidates, and this is in agreement with at least two previous studies in the western Mediterranean Sea which showed that growth rate of picoplankton could vary over a twofold range in response to cloud cover or nutrient availability (Jacquet *et al.*, 1998a; Vaulot and Partensky, 1992). The comparison for picoeukaryotes between the two studies is much more uncertain because this community is taxonomically much more diverse, and each species probably displays a specific relation between $RALS_{max/min}$ and division rate. Despite this caveat, we found a twofold variation in RALS in our study, whereas Vaulot and Marie only reported a 10% variation in $RALS_{max}/RALS_{min}$ from day to day, as well as higher absolute rates (Vaulot and Marie, 1999). Thus, this group seemed to grow much more vigorously in the Alboran Sea in winter than in the equatorial Pacific, maybe because nutrients are less limiting to growth in the former case. We also found that the $RALS_{max/min}$ ratio for this last group was very low on December 26 (as well as January 2–4),

which corresponded to those days with the lowest irradiance and which were among the windiest. As inferred from wind, temperature and salinity signatures registered for this day, vertical mixing was probably important (see Figures 3A and 3B). Provided that nutrients were not severely limiting for growth (samples from modified Atlantic waters), the low value for $RALS_{max/min}$ clearly suggests that light could be limiting for picoeukaryote growth this day, but not for the two prokaryotes for which $RALS_{max/min}$ remained constant. In contrast, $RALS_{max/min}$ was relatively high on December 30, a day with high solar irradiance and almost no wind, suggesting that the cells remained near the surface and had enough light to grow.

The observation of clear diel patterns for Chl fluorescence is in apparent contradiction with recent studies demonstrating that, in temperate waters, total chlorophyll fluorescence does not display any clear diel cycle, in contrast to equatorial and tropical areas (Dandonneau and Neveux, 1997; Claustre *et al.*, 1999). However, in temperate waters, bulk chlorophyll may be more related to nano- and microplankton (e.g. diatoms and dinoflagellates) that may not be as tightly phased to the daily cycle as picoplankton. In addition, the lack of diel patterns for bulk fluorescence might also be the result of differences in phasing of each population to the daily light cycle, so that the global signal does not display any 24-h periodicity. Chl fluorescence exhibited noisier diel variations with a lower amplitude than did scatter. The latter feature was observed elsewhere both in the Mediterranean Sea (Jacquet *et al.*, 1998a) and in the equatorial Pacific (Vaulot and Marie, 1999) and may be explained by the contrasting vertical distributions of the two parameters. In the equatorial Pacific mixed layer, RALS shows only a weak vertical stratification at all times of the day, in contrast to Chl fluorescence which displays very strong vertical gradients, especially in the middle of the day, due to the opposing effects of photoinhibition in surface waters and photoacclimation at depth (Vaulot and Marie, 1999). Since the situation is probably similar in the Alboran Sea, water advected to the surface from different depths or water masses would have almost similar RALS (but possibly *Prochlorococcus*) but very different Chl fluorescence, yielding a noisier signal for the latter parameter. For *Prochlorococcus*, the decrease in Chl fluorescence in the morning is probably related to photo-damage of photosynthetic reaction centres as previously hypothesized by Vaulot and Marie in the equatorial Pacific (Vaulot and Marie, 1999). The comparison of two days with different irradiance patterns (e.g. December 27 vs. January 6) indicates that the timing of maximum quenching followed that of the maximal irradiance as expected for such a short-term phenomenon (see Figure 9E, F). Although Chl fluorescence patterns for *Synechococcus* were very noisy, quenching could be also observed in some cases (typically on sunny days) as observed in the equatorial Pacific (Vaulot and Marie, 1999). The absence, in general, of quenching for *Synechococcus* has been interpreted as an indication of more efficient photoprotection for this organism (Vaulot and Marie, 1999). It is noteworthy here that we are well aware that sampling may have introduced bias when considering quenching of the different populations; changes in photochemical quenching have a timescale of a few minutes, and thus may be shorter than the time it takes to sample cells from the natural environment and arrest or lower their metabolic rate in the cooling device. It was however not possible to test (confirm or refute) such a feature from our data set. Mean Chl fluorescence of *Synechococcus* was in general little affected by irradiance level. However, a marked increase in fluorescence and in the magnitude of oscillations between December 27 and 28 clearly corresponded to a shift from nutrient-depleted Mediterranean waters to Atlantic waters, which could reflect an increase in cellular pigment concentration in response to nutrient enrichment [see (Cavender-Bares *et al.*, 1999) and references therein]. While picoeukaryote diel oscillations for Chl fluorescence were more marked than those of cyanobacteria, no evidence of quenching was observed as in previous studies (Jacquet *et al.*, 1998b; Vaulot and Marie, 1999). It was suggested that eukaryotes are less sensitive to light stress because of photoprotective mechanisms such as the xanthophyll cycle (Demming-Adams and Adams, 1992). Although we could observe different picoeukaryote populations, the clear diel pattern for the Chl fluorescence of the whole community suggests that all populations could display the same timing of growth and division in response to the daily cycle. In fact, in the laboratory, a range of picoeukaryotic species display similar cellular patterns when grown under identical light : dark conditions (Jacquet *et al.*, 2001b).

The dephasing observed here for the cell division of *Synechococcus* and picoeukaryotes (at dusk) vs. that of *Prochlorococcus* and cryptophytes (at night) differed from those reported in previous studies. For example, *Prochlorococcus* and picoeukaryotes were reported to display well-phased patterns and to divide at the same time in the equatorial Pacific (Blanchot *et al.*, 1997). Jacquet *et al.* showed that picoeukaryotes divided during daylight whereas *Synechococcus* divided later at night in surface waters of the northwestern Mediterranean Sea (Jacquet *et al.*, 1998a). In surface waters of the equatorial Pacific Ocean, Vaulot and Marie reported that *Synechococcus* divided first during daylight followed by *Prochlorococcus* at dusk and much later by picoeukaryotes in the middle of the night (Vaulot and Marie, 1999). Clearly, the phasing of

cell division is influenced by a variety of elements: genetic factors [e.g. prokaryotes vs. eukaryotes, (Jacquet *et al.*, 2001a,b)], light stress (Vaulot *et al.*, 1995) or nutrient depletion (Vaulot *et al.*, 1996).

### Conclusion and perspectives

Detailed analysis of diel patterns of autotrophic picoplankton remain an important clue to investigate in order to understand better the physiological status of the cells and the rate at which populations grow. Additionally, these questions are prerequisites to assess the long-term changes of populations and interpret the patterns observed in bulk measurements. In the present study, we described patterns of variability in abundance and cellular parameters of picophytoplankton and an attempt was made to analyse them. It appears clear that diel patterns of autotrophic populations are very complex since each population and each cellular parameter displays its own behaviour, partly modulated by light availability to the cells, nutrient level and mixing conditions. An interesting result of our study is that the growth rate of the populations, especially of the cyanobacteria, seems poorly affected in these ecosystems despite strong hydrological gradients compared to more stable systems such as those found in the tropical ocean. This clearly points out the fact that growth-rate regulation is related to other key parameters, such as light and nutrient levels. In the very dynamic area of the Alboran Sea, the question of deconvoluting environmental effects and diel variations of cellular properties in such a complex environment must be clearly answered using powerful statistical tools. The physiological component of the variability (diel patterns) will give us insights on population dynamics of the different groups and the environmental component will give us insights on the effects of physical and chemical factors on the distribution and abundance of the different autotrophs in the system. This issue is under investigation in a separate project.

## ACKNOWLEDGEMENTS

This work was supported by Frontal (France-JGOFS) and by contracts MAS3-CT95-0016 (MEDEA) and CT97-0128 (PROMOLEC) from the European Commission. S.J. benefited from a doctoral fellowship from the Ministère de la Recherche et de l'Enseignement Supérieur and from a short-term fellowship from 'la Société de Secours des Amis des Sciences' from the French Académie des Sciences. We want to thank P. Morin who kindly gave us access to nutrient data and to Frédéric Partensky, Richard Geider and some anonymous reviewers for their critical reading of a former version of this manuscript. Many thanks are addressed to all scientific participants and to the crew of the NO 'l'Atalante' for the excellent atmosphere prevailing on board during the cruise, especially on December 24 and 31.

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*Received on July 18, 2001; accepted on February 12, 2002*