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DIEL PATTERNS OF GROWTH AND DIVISION IN MARINE PICOPLANKTON IN CULTURE[1]

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The effect of a 12:12-h light:dark (LD) cycle on the phasing of several cell parameters was explored in a variety of marine picophytoplanktonic strains. These included the photosynthetic prokaryotes *Prochlorococcus* (strains MED 4, PCC 9511, and SS 120) and *Synechococcus* (strains ALMO 03, ROS 04, WH 7803, and WH 8103) and five picoeukaryotes (*Bathycoccus prasinos* Eikrem et Throndsen, *Bolidomonas pacifica* Guillou et Chrétiennot-Dinet, *Micromonas pusilla* Manton et Parke, *Pelagomonas calceolata* Andersen et Saunders, and *Pycnococcus provasolii* Guillard et al.). Flow cytometric analysis was used to determine the relationship between cell light scatter, pigment fluorescence, DNA (when possible), and the LD cycle in these organisms. As expected, growth and division were tightly coupled to the LD cycle for all of these strains. For both *Prochlorococcus* and picoeukaryotes, chl and intracellular carbon increased throughout the light period as estimated by chl fluorescence and light scatter, respectively. In response to cell division, these parameters decreased regularly during the early part of the dark period, a decrease that either continued throughout the dark period or stopped for the second half of the dark period. For *Synechococcus*, the decrease of chl and scatter occurred earlier (in the middle of the light period), and for some strains these cellular parameters remained constant throughout the dark period. The timing of division was very similar for all picoeukaryotes and occurred just before the subjective dusk, whereas it was more variable between the different *Prochlorococcus* and *Synechococcus* strains. The burst of division for *Prochlorococcus* SS 120 and PCC 9511 was recorded at the subjective dusk, whereas the MED 4 strain divided later at night. *Synechococcus* ALMO 03, ROS 04, and WH 7803, which have a low phycourobilin to phycoerythrobilin (PUB:PEB) ratio, divided earlier, and their division was restricted to the light period. In contrast, the high PUB:PEB *Synechococcus* strain WH 8103 divided preferentially at night. There was a weak linear relationship between the $FALS_{max}$:$FALS_{min}$ ratio and growth rate calculated from cell counts ($r = 0.83$, $n = 11$, $P < 0.05$). Because of the significance of picoplanktonic populations in marine systems, these results should help to interpret diel variations in oceanic optical properties in regions where picoplankton dominates.

Key index words: **cell cycle; diel rhythms; picoeukaryotes; *Prochlorococcus*; size; *Synechococcus***

Abbreviations: **FALS, forward-angle light scatter; FCM, flow cytometry; PE: phycoerythrin; PUB:PEB, phycourobilin:phycoerythrobilin ratio; RALS, right-angle light scatter**

In natural environments, phytoplankton are influenced by a variety of environmental factors, such as nutrient availability, temperature, mixing, and irradiance level. Among these, the daily alternation of light and darkness is undoubtedly a very important external stimulus. Diel periodicity has been documented for phytoplankton biomass and productivity (Prézelin 1992), nutrient uptake (Chisholm 1981), cell division (Nelson and Brand 1979), or gene expression (Liu et al. 1996). In oligotrophic areas, variations occurring at the daily scale are probably more relevant than those at monthly, seasonal, or annual scales. In particular, clear diel cycles of bulk oceanographic parameters related to phytoplankton such as beam attenuation or chl fluorescence have been reported in the tropical Pacific (Siegel et al. 1989), the North Atlantic (Gardner et al. 1995), and the equatorial Pacific (Claustre et al. 1999). Diel variations in optical properties also have been used to estimate oceanic primary production (Siegel et al. 1989).

The picoplanktonic fraction of marine phototrophs, *Prochlorococcus*, *Synechococcus*, and the picoeukaryotes, is now recognized to constitute the bulk of chl and carbon biomass in both intertropical and temperate oligotrophic areas (Li et al. 1992, Campbell and Vaulot 1993). *Prochlorococcus* and *Synechococcus* are closely related cyanobacteria with different sizes and light-harvesting antenna systems that enable them to occupy different ecological niches (for a review, see Partensky et al. 1999a). In contrast, picoeukaryotes constitute a much wider taxonomic assemblage, of which the diversity is only beginning to be revealed (Potter et al. 1997). Recent studies have shown that these picophytoplanktonic populations display clear diel patterns in the field.

Cell parameters such as DNA or carbon content and chl fluorescence of *Prochlorococcus* and *Synechococcus* are highly synchronized to the daily cycle, and physiological processes such as cell division take place every day during a very short time window in a variety of environments, including the equatorial Pacific, the tropical Atlantic, the Mediterranean Sea, or the Arabian Sea (e.g. Vaulot et al. 1995, Jacquet et al. 1998a). Phased cell division in the field is of particular interest

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because it allows assessment of population dynamics without the need to incubate samples. In particular, determination over a complete diel cycle in the variation of the percentage of cells in a terminal event of the cell cycle provides estimates of absolute growth rates (Carpenter and Chang 1988).

The causes of diel periodicity of cellular parameters are still poorly understood. Two major hypotheses have been advanced to explain the cell division cycle synchrony of phototrophic organisms: a direct control by light over a part of the cell cycle (Spudich and Sager 1980) or the existence of an entrainable biological clock whose diel periodicity can keep many cell processes phased over light:dark (LD) cycles (Edmunds and Adams 1981). Both mechanisms probably coexist. The latter hypothesis has been clearly demonstrated for *Synechococcus* (Sweeney and Borgese 1989) and its genetic basis uncovered (Ishiura et al. 1998). Nevertheless, sensors responsible for the entrainment of cell cycle remain to be identified.

In the present study, short-term variations (≤1 h) of cell concentration, light scatter, pigment fluorescence, and cellular DNA content were investigated by flow cytometry (FCM) using batch cultures acclimated to a 12:12-h light:dark (LD) cycle for a variety of photosynthetic prokaryotic and eukaryotic strains of picoplanktonic size. The main goals of this study were to determine 1) the specific oscillation patterns for the three major groups of picoplanktonic phototrophs, 2) the relationships between the patterns of the different cell parameters (size, pigment fluorescence, cell cycle) for a given phototroph, and 3) the relationship between the imposed LD cycle and the cellular patterns. These data should help to interpret patterns that have been observed in the field.

MATERIALS AND METHODS

Cultures. Characteristics of marine strains used in this study are reported in Table 1. *Prochlorococcus* strains SS 120 (CCMP 1375) and MED 4 (CCMP 1378) were obtained courtesy of Dr. L. Moore and Prof. S.W. Chisholm (MIT, Cambridge, MA). PCC 9511 is an axenic *Prochlorococcus* strain (Rippka et al. 2000). *Synechococcus* strains WH 8103 and WH 7803 were kindly provided by Drs. D. Scanlan (Warwick University, Coventry, UK) and B. Binder (University of Georgia, Athens, GA), respectively.

Growth conditions. Batch cultures of prokaryotes and eukaryotes were grown, respectively, in PCR-S11 (Rippka et al. 2000) and in K medium (Keller et al. 1987) made from filtered seawater aged for 2 months. Cultures were maintained in 500 to 1000 mL polyethylene Nalgene flasks (Bioblock, Illkirch, France) under blue light provided by four Daylight TLD $18W/82_7$ fluorescent bulbs (Philips), wrapped with "Moonlight Blue" filters (Lee Filters no. 183, Panavision, France). Light intensity was measured inside flasks filled with filtered seawater using a LI-COR quanta-meter (Li-cor Ltd., Lincoln, NE) equipped with a 4π spherical sensor. A 12:12-h LD cycle, with irradiance fixed at 25 μmol quanta·m^{-2}·s^{-1} during the light period, was applied to all strains. Average temperature was 20 ± 1° C. This temperature was suitable for near optimal growth for all studied organisms. Cultures were acclimated to these conditions for at least 2 weeks, and sampling began 3 to 5 days after the last dilution of the cultures (dilutions took place about once a week).

Culture sampling. Exponentially growing cultures were sampled every hour during 48 h. Sampling was performed automatically using a computer-controlled peristaltic pump (Masterflex, Bioblock, Illkirch, France) and a custom-designed fraction collector (modified from Jacquet et al. 1998b; details can be found at http://www.sb-roscoff.fr/Phyto/SJ_sampler_98.html). The tubing system was equilibrated during 2 days before the experiment to eliminate possible toxic effects. Samples were kept until analysis or fixation in a large Plexiglas tank filled with circulating water at 4° C from a Minichiller system (Bioblock). Storage at 4° C for up to 10 h was previously shown to result in minimal effects on parameters measured by FCM, such as abundance, right-angle light scatter (RALS; a proxy for cell size), pigment fluorescence, and DNA histograms (Jacquet et al. 1998b).

Sample processing. Samples were divided into two aliquots. The first aliquot was analyzed fresh by FCM, generally after dilution with 0.2-μm filtered seawater to avoid coincidence problems associated with high count rates. Efforts were made to reduce the lag interval between sampling and analysis for samples collected at night (<8 h). The second aliquot was fixed for 15 min with either glutaraldehyde for prokaryotes (0.25% final concentration) or paraformaldehyde for eukaryotes (1% final), frozen in liquid nitrogen, and stored at −80° C for delayed cell cycle analysis. Before analysis, this aliquot was thawed and incubated for 1 h at 37° C in the presence of 0.1 g·L^{-1} of a mixture of RNase A and B (R-4875 and R-5750, 1:1, w:w, Sigma, Saint-Quentin Fallavier, France). After dilution (if necessary) with 0.2-μm filtered seawater, samples were stained with SYBR Green I (Molecular Probes Inc., Eugene, OR, 1/10,000 final concentration) for at least 10 min (Marie et al. 1997).

Table 1. Characteristics of the different marine strains and species used in this study.

Class	Genus and/or species	Strain	RCC	CCMP	Unialgal/ clonal	Origin	Depth (m)	Phenotype
Cyanophyceae	*Prochlorococcus*	MED 4	153	1378	U	NW Mediterranean Sea	5	Low chl *b/a*
—	*Prochlorococcus marinus* subsp *pastoris*	PCC 9511	168		C			Low chl *b/a*
—	*Prochlorococcus marinus*	SS 120	156	1375	U	Sargasso Sea	120	High chl *b/a*
—	*Synechococcus*	ALMO 03	43		U	Alboran Sea	Surface	Low PUB:PEB
—	*Synechococcus*	ROS 04	32		U	English Channel	Surface	Low PUB:PEB
—	*Synechococcus*	WH 7803	28	1334	U	N Atlantic	25	Low PUB:PEB
—	*Synechococcus*	WH 8103	29		U	Sargasso Sea	Surface	High PUB:PEB
Prasinophyceae	*Bathycoccus prasinos*	ALMO 02	113		U	Alboran Sea	Surface	
Prasinophyceae	*Micromonas pusilla*		114	490	U	n. a.	n. a.	
Prasinophyceae	*Pycnococcus provasolii*		117	1203	U	W Atlantic	30	
Bolidophyceae	*Bolidomonas pacifica*	OLI31SA	201		U	Equatorial Pacific	15	
Pelagophyceae	*Pelagomonas calceolata*		100	1214	U	Equatorial Pacific	110	

n. a., data not available; CCMP, Culture Collection of Marine Phytoplankton; PCC, Pasteur Culture Collection; RCC, Roscoff Culture Collection.

Flow cytometric and data analysis. Samples were analyzed with a FACSort flow cytometer (Becton Dickinson, San Jose, CA) that provides two size-related parameters: the forward-angle light scatter (FALS) and RALS and three fluorescence signals referred to as "green" (530 ± 15 nm), "orange" (585 ± 21 nm), and "red" (>650 nm) fluorescence related, respectively, to DNA, phycoerythrin (PE), and chl contents of the cells. Cell concentration, FALS, RALS, PE, and chl fluorescences were acquired on fresh samples, and cell parameters were normalized to 0.95-μm fluorescent beads (Polyscience, Inc., Warrington, PA). On SYBR Green I stained samples, green fluorescence from the DNA-dye complex was measured both on logarithmic and linear scales, as required for cell cycle analysis (Marie et al. 1997). It was not possible to obtain reliable cell cycle data for either the low phycourobilin:phycoerythrobilin (PUB:PEB) *Synechococcus* strains or for most eukaryotic species (with the notable exception of *Bathycoccus prasinos*) because of either poor dye penetration or interference with a large number of heterotrophic bacteria. Data were collected in list-mode files and then analyzed using the custom-designed freeware CYTOWIN (modified from Vaulot 1989; available at http://www.sb-roscoff. fr/Phyto/cyto.html). Cell cycle analyses were performed using MultiCYCLE (P.S. Rabinovitch, Phoenix Flow Systems, San Diego, CA).

Specific growth rate. Division rate was estimated from cell cycle data, when available, using the formula of Carpenter and Chang (1988):

$$\mu_{CC} = \frac{\sum_{i=1}^{n} \ln[1 + f_S(t_i) + f_{G_2}(t_i)]}{n \times (T_S + T_{G_2})} \times 24 \quad (1)$$

where μ_{CC} is an estimate of the division rate (day^{-1}); n is the number of samples collected at fixed intervals during one subjective day; $T_S + T_{G2}$ (h) is the sum of the duration of S and G_2 phases, computed as twice the delay between the peaks of cells in these phase $[2(t_{G2max} - t_{Smax})]$; and $f_S(t_i)$ and $f_{G2}(t_i)$ are the fractions of cells in S and G_2 phases at time t_i (Table 2).

When no cell cycle data were available, growth rate was estimated from cell abundance using

$$\mu_{NB} = \frac{\mathrm{Ln}(N(t_2)/N(t_1))}{(t_2 - t_1)} \quad (2)$$

where $N(t)$ is the average cell concentration ($n = 3$) at time t and t_1 and t_2 correspond to the beginning and the end of a 24-h sampling period when no division occurs, that is, in early morn-

TABLE 2. Growth rate calculated from cell number variation (μ_{NB}) and from cell cycle (μ_{CC}) over a diel cycle.

Strains or species	μ_{NB}	μ_{CC}	T_S	T_{G_2+M}	T_g
Prokaryotes					
Prochlorococcus MED 4	0.6	0.48	6.5	4.5	35
Prochlorococcus PCC 9511	0.55	0.63	3	3	26
Prochlorococcus SS 120	0.4	0.57	4	5	29
Synechococcus ALMO 03	0.49	n. a.	n. a.	n. a.	34
Synechococcus ROS 04	0.33	n. a.	n. a.	n. a.	50
Synechococcus WH 7803	0.17	n. a.	n. a.	n. a.	98
Synechococcus WH 8103	0.48	0.54	3.5	2.5	31
Eukaryotes					
Bathycoccus prasinos	0.23	0.49	6.5	2.5	34
Bolidomonas pacifica	0.91	n. a.	n. a.	n. a.	18
Micromonas pusilla	0.37	n. a.	n. a.	n. a.	45
Pelagomonas calceolata	0.5	n. a.	n. a.	n. a.	33
Pycnococcus provasolii	0.51	n. a.	n. a.	n. a.	33

T_S and T_{G_2+M} are the duration of active cell cycle phases S and G_2 as obtained from Carpenter and Chang's model (1988). Generation time (T_g), calculated using the formula $24 \times (\ln 2/\mu)$, is estimated preferentially to μ_{CC} when available otherwise from μ_{NB}. Division rates and durations are given in d^{-1} and h respectively. Cultures were grown in batch under 12:12-h LD cycle (25 μmol quanta·m^{-2}·s^{-1}) at 20° C. n. a., not available.

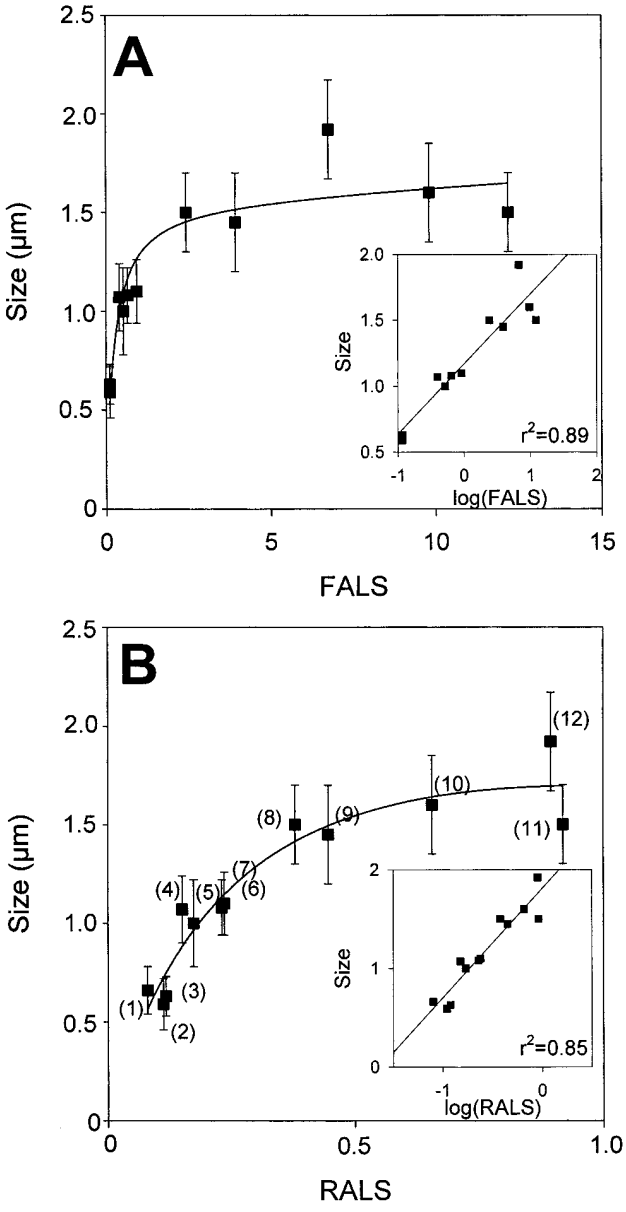

FIG. 1. Relationships between cell size (mean value and SD assessed by confocal microscopy and image analysis) and FALS (A) or RALS (B) for cultures of the picoplankters entrained by LD cycle sampled at "light on." Insets correspond to relationships between size and log of parameters (see Materials and Methods for equations). Cell size corresponds to average Feret diameter for prokaryotes and maximal length of the chloroplast for eukaryotes. FALS and RALS are normalized values to 0.95-μm-diameter spherical beads. Strain designation with number of measurements (n) are as follows: 1, PCC 9511 (n = 843); 2, SS 120 (n = 1342); 3, MED 4 (n = 1382); 4, WH 8103 (n = 788); 5, ROS 04 (n = 747); 6, WH 7803 (n = 57); 7, ALMO 03 (n = 122); 8, *Pycnococcus provasolii* (n = 321); 9, *Bathycoccus prasinos* (n = 362); 10, *Micromonas pusilla* (n = 107); 11, *Bolidomonas pacifica* (n = 154), and 12, *Pelagomonas calceolata* (n = 293).

ing when most of the cells are in the G_1 phase (Table 2). Dilution and pipetting errors and variation in the rate of the FCM sample delivery (due to possible temperature and hygrometric fluctuations) may introduce uncertainties in cell concentration estimates and, as a consequence, in estimates of μ_{NB} (see also Discussion).

Calibration of FCM parameters by confocal microscopy. Cell size was determined with a confocal laser scanning microscope (CLSM, Fluoview, Olympus Optical Co., Tokyo, Japan) followed by image analysis. Cells were fixed with glutaraldehyde (0.25% final concentration) and then filtered on 0.2-μm pore size inorganic membrane filter disks (Anodisc, Whatman, Maidstone, UK). The filter disk was placed between a glass slide and a 22 × 40-mm coverslip in a 20-μL mixture (50%–50%) of normal PBS (Sigma) and bidistilled glycerol (d = 1.26, Fisher Scientific, Elancourt, France). The inverted CLSM was equipped with an argon-krypton continuous laser (model 643R-OLYM-A03, 20 mW, Omnichrome, Melles Griot Laser Group, Carlsbad, CA). An Uplan Fi ×60 objective (numerical aperture, 1.25) was used with a pinhole size equal to the corresponding Airy diffraction disk. Laser light excitation (488 and 567 nm) could be tuned at 6%, 20%, or 50% of maximum power. Particular care was taken not to saturate the fluorescence signal by modulating the laser power or the sensitivity of the photomultiplier. Cells were observed according to their natural pigment fluorescence. For prokaryotes, thylakoids surround the cell and cell size can be obtained directly from the red fluorescence image. For eukaryotes, the chloroplast does not occupy the whole cell; therefore, the cell outline was approximated by the smallest circle surrounding the chloroplast. In all cases, cell diameter was estimated as the Feret diameter (d) given by the Image Tools software (version 1.27, T. Wilcox, available at http://www.uthscsa.edu/dig/download.html) after appropriate calibration with a micrometer. Calibrated fluorescent beads (0.95, 1.98, and 3.15 μm in diameter) were used to establish the accuracy of the method. The following relationships were obtained for the picoplankton strains (Fig. 1):

$$d = 0.53 \cdot \log(\text{FALS}) + 1.17 \quad (r^2 = 0.89,\ n = 11,\ P < 0.01)$$

$$d = 1.10 \cdot \log(\text{RALS}) + 1.80 \quad (r^2 = 0.85,\ n = 12,\ P < 0.01)$$

These equations were used to interpret the relative changes in FALS or RALS in terms of cell size changes (Table 3).

RESULTS

Prochlorococcus. Three strains of *Prochlorococcus* were examined: MED 4 and PCC 9511 (axenic), both characteristic from surface waters, and SS 120, characteristic from deep waters. The major increase in cell concentration occurred during the first part of the subjective night (Fig. 2, A, F, and K). During the light period, cell numbers remained quite stable or even decreased. Size-related parameters, FALS and RALS, were subjected to strong oscillations. Minimum and maximum relative values were recorded around the dark-light and light-dark transitions, respectively (Fig. 2, B, C, G, H, and L). The daily patterns for chl fluorescence signal (Fig. 2, D, I, and M) paralleled those of scatter (i.e. a regular increase during the light period and a decrease at night) but with smaller amplitudes. Cell cycle was well phased to the LD cycle (Fig. 2, E, J, and N). It resembled that typically observed in eukaryotes, that is, a discrete DNA synthesis S phase separates two well-defined G_1 and G_2 phases. The percentage of cells in the S phase of the cell cycle began to increase in the middle of the light period with a concomitant decrease in the percentage of cells in G_1. Maxima in S were reached 3 to 5 h before darkness, and the burst of division as inferred from the peak of cells in G_2 occurred early at night. A second minor peak of cells in S phase was often recorded after the light-to-dark transition—but not for G_2. Estimation of phase duration and division rate with Carpenter and Chang's model (1988) revealed in most cases longer duration for both S and G_2 phases and a larger generation time for MED 4 than for the other two strains (Table 2).

Synechococcus. The *Synechococcus* strains analyzed were representative from both coastal and oceanic regions (Table 1). WH 7803, ROS 04, and ALMO 03 have a low PUB:PEB ratio, whereas WH 8103 has a high PUB:PEB ratio (not shown). Clear daily variations were observed for all cellular parameters (Fig. 3). The major increase in cell concentration was recorded in the second part of the light period for ALMO 03 (Fig. 3E), around the LD transition for ROS 04 and WH 7803 (Fig. 3, A and I), and restricted to the dark period for WH 8103, at least during the first diel cycle (Fig. 3M). FALS and RALS were clearly phased to the LD cycle, but the patterns differed significantly between strains. The maxima for light scatter were recorded during daylight for both ROS 04 and ALMO 03 and at the light-to-dark transition or slightly after (ca. 1 h after) for WH 7803 and WH 8103, respectively. Scatter began to increase when the light was turned on except for ROS 04, for which RALS (but not FALS) was delayed to 2 h after the beginning of the light period (Fig. 3, B and C). Past the daily maximum, scatter patterns differed clearly between strains. In ROS 04 and ALMO 03, two phases were recorded: First RALS decreased until the end of the light period and then it remained constant during darkness. Such behavior also was recorded for FALS of ROS 04 but not for ALMO 03. For the two other strains, WH 7803 and WH 8103, both FALS and RALS decreased throughout the dark period.

Chl and PE fluorescence displayed clear diel patterns with an increase during most of the daylight period. Some differences appeared in the last part of the light period and at night. In WH 8103, both chl and PE fluorescence increased during daylight and then decreased regularly during the dark period until reexposure to light (Fig. 3P). For both ALMO 03 and WH 7803, chl and PE fluorescence remained nearly constant or even increased during darkness (Fig. 3, H and L). In ROS 04, chl fluorescence was characterized by a two-step decrease with a slope change at the LD transition, whereas PE fluorescence decreased rapidly and regularly all night long (Fig. 3D). This behavior suggested two different regulatory mechanisms for chl and PE. Reliable cell cycle data could only be obtained for WH 8103 (Fig. 3Q). Its cell cycle was similar to that of *Prochlorococcus*, with discrete phases. Entry in S occurred in the middle of the light period, and the maximum was recorded around the LD transition. The peak of cells in G_2 was recorded 3 to 4 h after that of S phase. The persistence of a significant fraction of

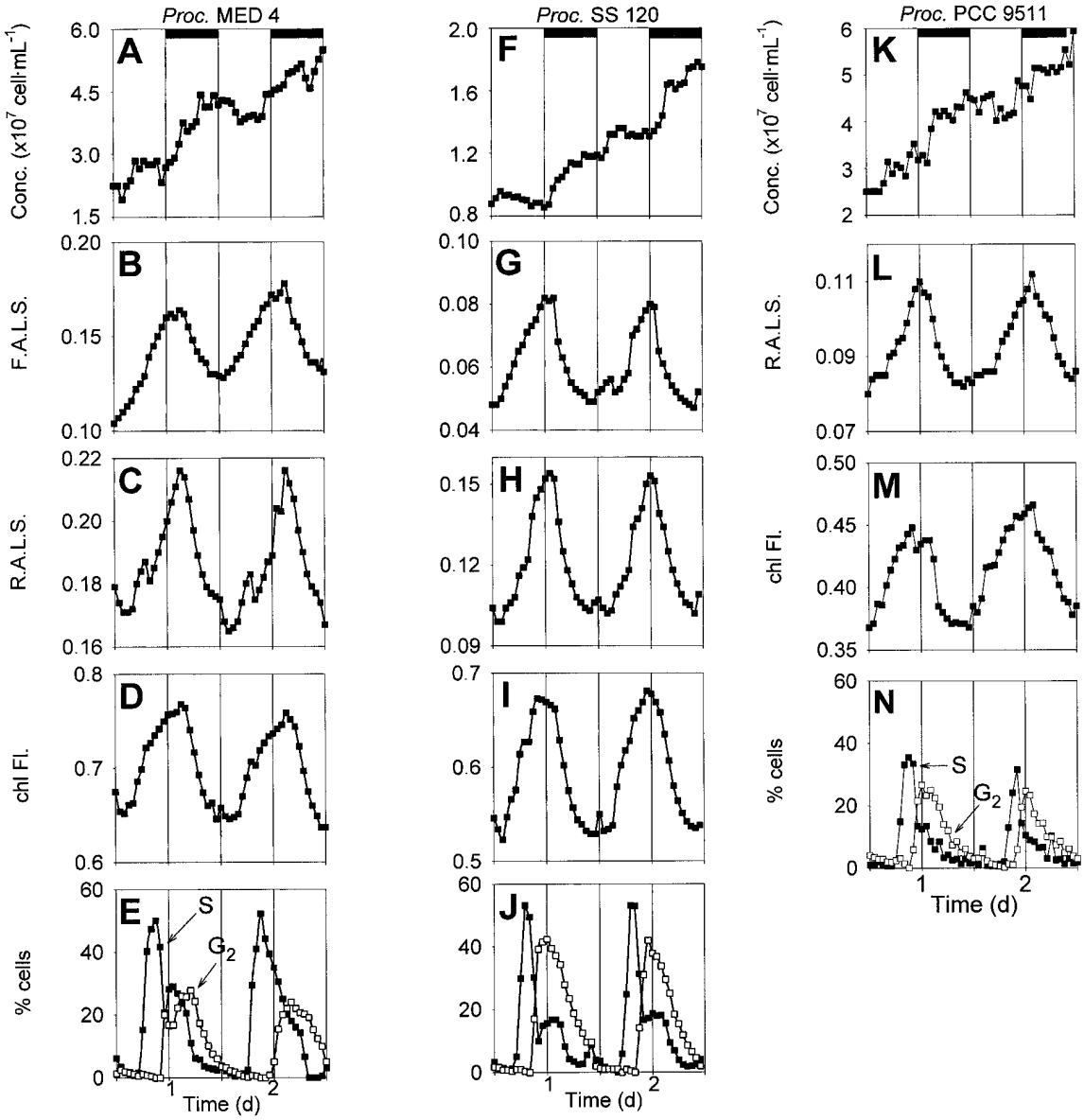

FIG. 2. Growth of *Prochlorococcus* strains MED 4, SS 120, and PCC 9511 under 12:12-h LD cycle with constant irradiance fixed at 25 μmol quanta·m^{-2}·s^{-1}. Cell concentration (A, F, and K), FALS (B and G), RALS (C, H, and L), chl fluorescence (D, I, and M), and percentage of cells in S and G_2 phases of the cell cycle (E, J, and N) are shown. Dark bars symbolize periods of darkness. No data were available for PCC 9511 FALS because of FCM problem.

cells in G_2 throughout the LD cycle (except when S was maximum, Fig. 3Q, but this is probably a computational artifact linked to cell cycle analysis, as G_2 cells are difficult to discriminate from cells in late S) was in agreement with previous observations of low and persisting levels of dividing cells throughout the day (e.g. Binder and Chisholm 1995). Phase duration and growth rate estimation were quite similar to those recorded for PCC 9511 and SS 120, respectively (Table 2).

Picoeukaryotes. The picoeukaryote species used in this study were chosen to represent different marine systems (Table 1). Each species displayed clear diel patterns of FALS, RALS, and chl fluorescence (Fig. 4), and division occurred just before the subjective dusk. Minimum values for FALS, RALS, and chl fluorescence were recorded approximately at the dark-to-light transition and maximum values 2 to 3 h before darkness (Fig. 4). Chl fluorescence patterns evolved similarly to FALS and RALS, that is, there was a clear and regular increase during the daylight period and a decrease at night (Fig. 4). Patterns obtained for the cell cycle of *B. prasinos* were in agreement with results recorded for the other parameters (Fig. 4I). The fraction of cells in S increased 2 h after light onset and peaked in the middle of the light period (Fig. 4I). The G_2+M peak occurred 4 h later. Phase duration

TABLE 3. Maximum to minimum ratios of FALS and RALS over a diel cycle and corresponding size variations measured from the nonlinear relationships drawn on Figure 1.

Strains	$FALS_{max}/FALS_{min}$	Δsize-FALS (%)	$RALS_{max}/RALS_{min}$	Δsize-RALS (%)	Size (μm) AM	Size (μm) PM
Prokaryotes						
Prochlorococcus MED 4	1.6	12	1.3	14	0.63	0.72
Prochlorococcus PCC 9511			1.4	17	0.59	0.69
Prochlorococcus SS 120	1.7	14	1.5	23	0.66	0.82
Synechococcus ALMO 03	1.4	9	1.3	13	1.00	1.13
Synechococcus ROS 04	1.3	8	1.1	7	1.07	1.15
Synechococcus WH 7803	1.1	3	1.1	4	1.08	1.12
Synechococcus WH 8103	1.8	15	1.2	10	1.10	1.27
Eukaryotes						
Bathycoccus prasinos	1.5	12	1.2	11	1.50	1.67
Bolidomonas pacifica	2.6	26	1.3	15	1.50	1.73
Micromonas pusilla	1.8	16	1.3	12	1.45	1.68
Pelagomonas calceolata	1.9	17	1.3	14	1.92	2.25
Pyconococcus provasolii	1.9	17	1.5	20	1.60	1.92

Size referred to as AM corresponds to that really measured at the light onset by confocal microscopy followed by image analysis and that referred to as PM is inferred from the RALS variation.

and growth rate estimation were in the same range as those reported for *Prochlorococcus* MED 4 (Table 2).

Relation between size increase and division rate. When cell division is restricted to a precise period of the day and cells are well phased, it has been shown that the relationship between maximum and minimum values of scatters can be empirically related to growth rates (e.g. Binder et al. 1996). All our data pooled together

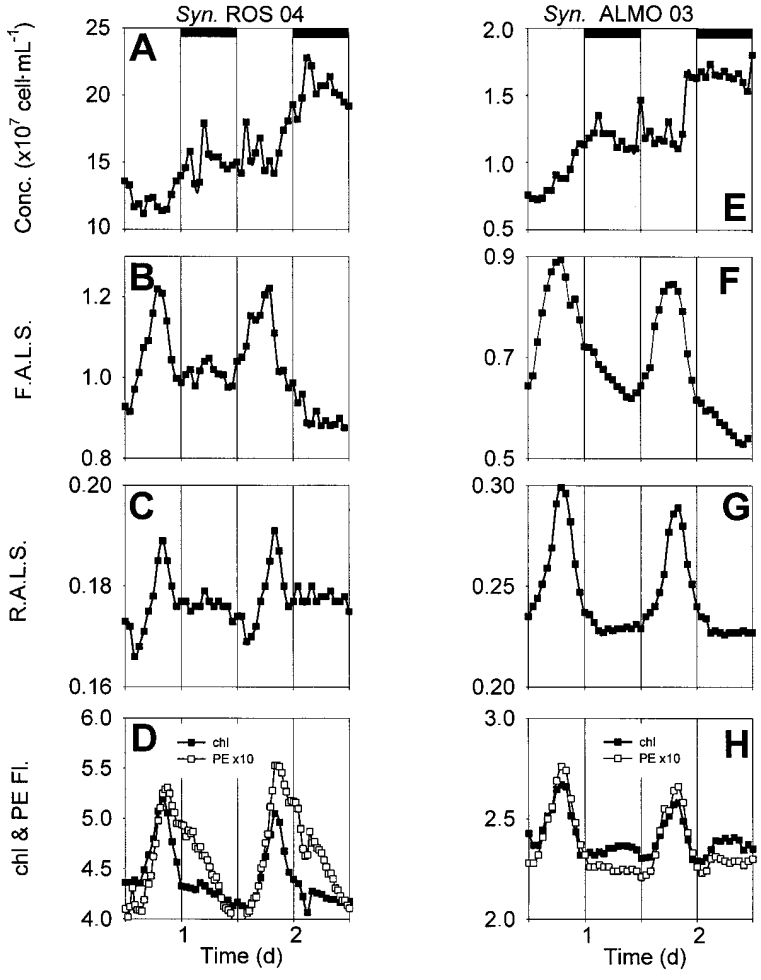

FIG. 3. Growth of *Synechococcus* strains ROS 04, ALMO 03, WH 7803, and WH 8103 under 12:12-h LD cycle with constant irradiance fixed at 25 μmol quanta·m^{-2}·s^{-1}. Cell concentration (A, E, I, and M), FALS (B, F, J, and N), RALS (C, G, K, and O), chl and PE fluorescence (D, H, L, and P), and percentage of cells in S and G_2 phases of the cell cycle of WH 8103 (Q) are shown. Dark bars symbolize periods of darkness.

yielded a linear relationship between μNB and $FALS_{max}$:$FALS_{min}$ ($r = 0.83$, $n = 11$, $P < 0.01$; Fig. 5) but not with $RALS_{max}$:$RALS_{min}$ (data not shown).

DISCUSSION

Although synchronization of oceanic populations in the field by the natural diel cycle has been known for a long time (Gough 1905) and investigated in the 1970s (e.g. Smayda 1975), it is only in the past 10 years that the ubiquity of this phenomenon has surfaced. Synchronization is especially easy to follow for picoplanktonic populations that can be monitored by FCM (e.g. Vaulot et al. 1995, DuRand and Olson 1996). However, to date, no laboratory study has been undertaken to compare patterns observed in major groups of picoplankton under similar experimental conditions.

The present work demonstrates that, broadly speaking, there is little variability in growth and division patterns across all picoplankton groups. In particular, increase in scatter and pigment fluorescence occurs almost only during the light period and is triggered by the onset of light. Scatter is a good proxy for cell size (Fig. 1) and cell carbon (Stramski et al. 1995, DuRand and Olson 1998), and therefore our data suggest that light is required for cell growth. This is especially visible in *Synechococcus* strains (e.g. Fig. 3G): Because division occurs relatively early and stops in the initial part of the dark period, scatter becomes constant early at night and increases again only at the dark-to-light transition. The major decrease in cell size is clearly linked to division. Once cells have divided (i.e. when no cells are left in G_2) and remain in the dark, their size (or carbon content) continue to decrease, probably due

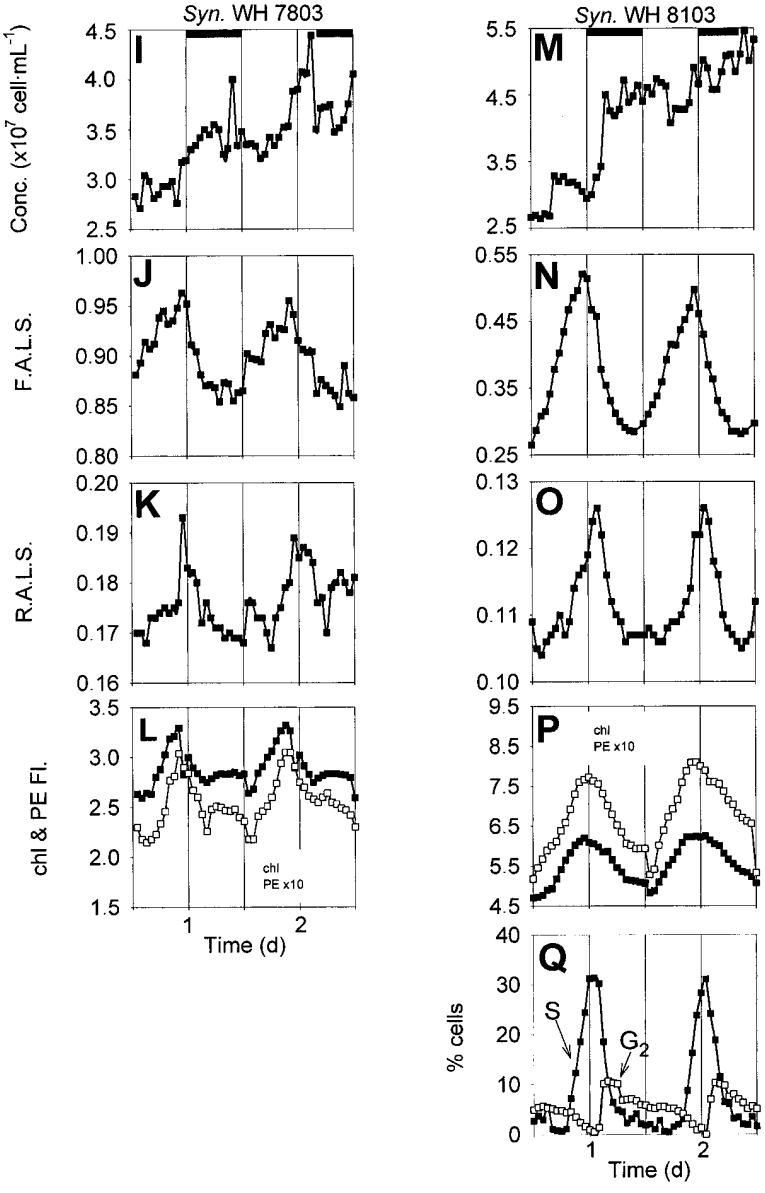

FIG. 3. *Continued.*

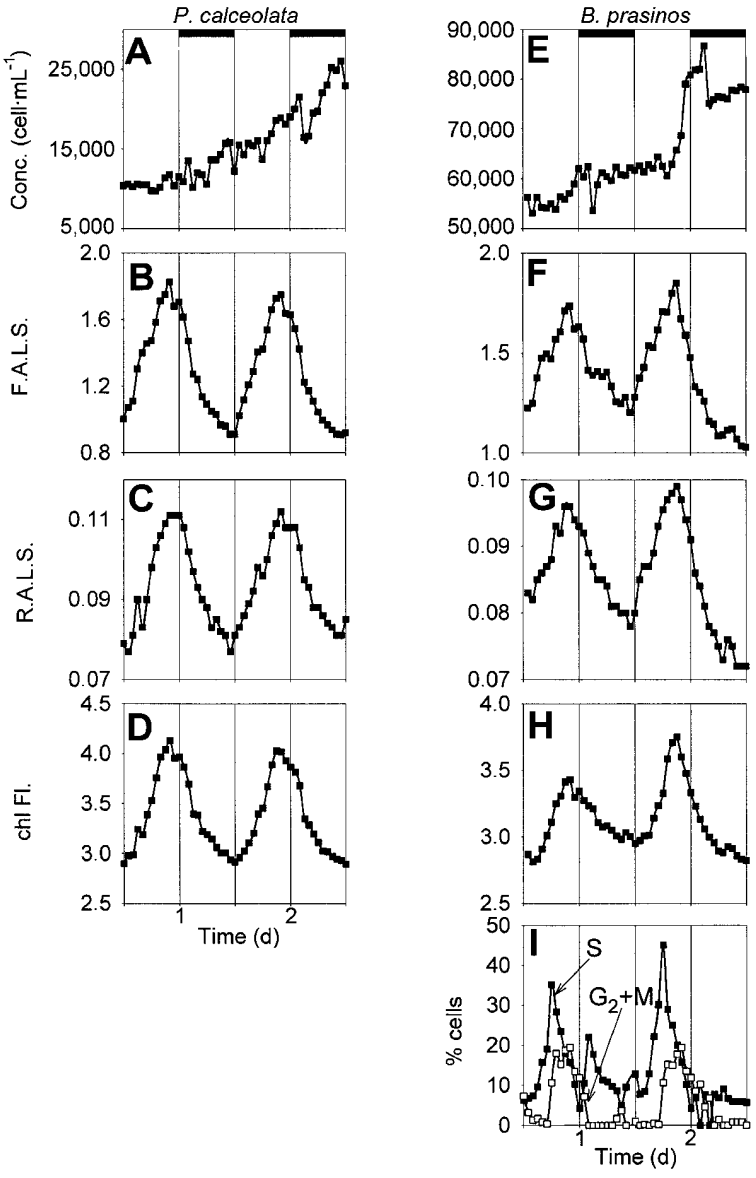

FIG. 4. Growth of eukaryotic species *Pelagomonas calceolata*, *Bathycoccus prasinos*, *Micromonas pusilla*, *Pycnococcus provasolii*, and *Bolidomonas pacifica* under 12:12-h LD cycle with constant irradiance fixed at 25 μmol quanta·m^{-2}·s^{-1}. Cell concentration (A, E, J, N, and R), FALS (B, F, K, O, and S), RALS (C, G, L, P, and T), chl fluorescence (D, H, M, Q, and U), and percentage of cells in S and G_2 phases (I) of the cell cycle of *Bathycoccus* are shown. Dark bars symbolize periods of darkness.

to respiration. It is noteworthy that in *Prochlorococcus*, the scatter of the cells that are transferred to complete darkness keeps decreasing for more than 1 day after division has stopped but eventually reaches an asymptotic value (Jacquet et al. 2001). The behavior of pigment fluorescence is in general parallel to that observed for scatter. As in our experiments, the light intensity used was probably not inhibitory (e.g. for *Prochlorococcus*)—no quenching occurred as observed, for example, in oceanic surface populations (Vaulot and Marie 1999)—and red fluorescence was probably mainly related to cell pigment content. Our data suggest that pigment synthesis and cell growth are probably tightly linked.

For all the organisms we examined, both prokaryotic and eukaryotic, the DNA replication phase S was always restricted to a fraction of the cell cycle, delimited by well-defined G_1 and G_2-like phases. In particular, we never observed continuous DNA replication or multiple copies of the chromosome, as reported by Binder and Chisholm (1995) in one of the strains we examined: WH 7803. This difference could either be due to our culture conditions or to actual genetic differences between the two cultures, although they have the same strain name. In all strains, division was restricted to the day-night transition, except for some *Synechococcus* strains for which it occurred earlier (see below). Interestingly, two successive S maxima occurred in some *Prochlorococcus* strains (Fig. 2), a phenomenon previously observed (Shalapyonok et al. 1998). Here, the second round of replication could be due to a partial asynchrony of the population, with two cohorts that replicate DNA slightly out of phase. Alternatively, some cells could undergo two successive

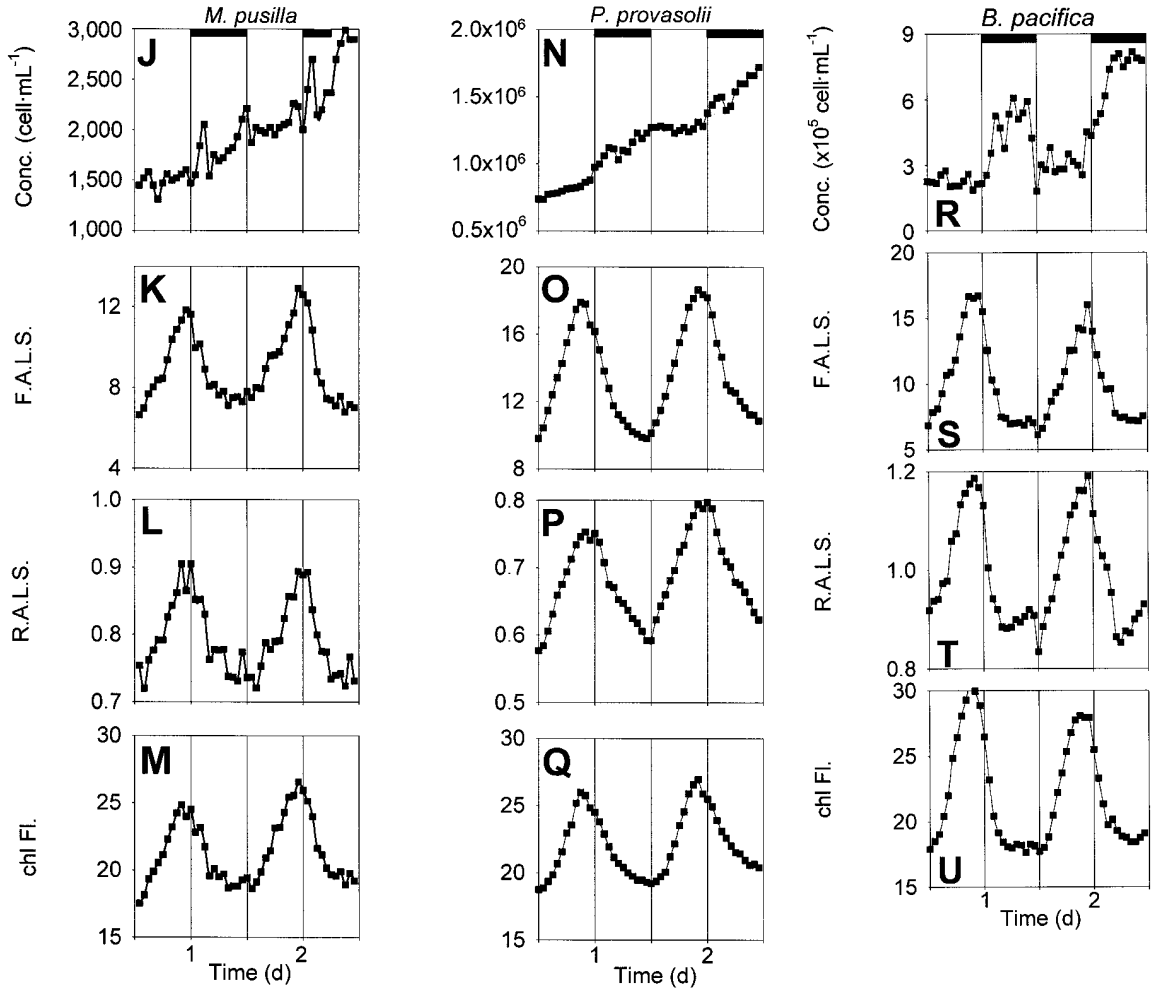

FIG. 4. *Continued.*

rounds of DNA replication and division despite the fact that the overall population divided less that once a day, in contrast to what was observed by Shalapyonok et al. (1998).

The absence of very marked differences in growth and division patterns in picoplankton is surprising in view of the large phylogenetic range examined from cyanobacteria to Prasinophytes, Pelagophytes, and Bolidophyceae. In fact, among larger sized groups such as diatoms or dinoflagellates, much more variability is observed; some diatoms, for example, divide early in the day (Chisholm 1981). *Synechococcus* was the only picoplankter exhibiting a wide range of phasing for division (see also Table 6). For this genus, timing of division appears linked to accessory pigment content of the cells because low and high PUB:PEB strains divided during daylight and at night, respectively. It is also noteworthy that within the low PUB:PEB, coastal strains ROS 04 and ALMO 03 divided earlier than the more oceanic representative WH 7803. These results are consistent with the fact that these genetically distinct strains may respond differently to their light environment (Binder and Chisholm 1995). For example, different strains may require different integrated light doses to initiate the division processes. Another unique characteristic of the low PUB strains was to stop division almost immediately in the dark, as suggested by the sudden change in the slope of scatters (see ROS 04 and ALMO 03 in Fig. 3, B, C, and G). There could be in these low PUB strains an absolute need of light to complete division as suggested by earlier studies (Armbrust et al. 1989, Binder and Chisholm 1995), in contrast to most photosynthetic unicells such as *Prochlorococcus* or diatoms (Vaulot et al. 1986, Jacquet et al. 2001). Clearly, more experimental work is needed to test a potential relationship between phenotypic characters such as the PUB:PEB ratio and the timing of cell division.

How do these patterns in culture compare with those observed in the field? Tables 4, 5, and 6 show a striking similarity between the two sets of observations. Broadly speaking, *Synechococcus* divides in general first (and displays more variability), followed by *Prochlorococcus*, followed by eukaryotes (the dephasing

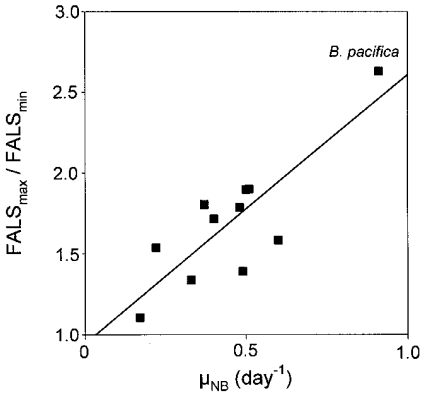

FIG. 5. Relationship between the ratio $FALS_{max}$:$FALS_{min}$ and growth rate calculated from cell numbers (μ_{NB}) ($r = 0.83$, $n = 11$, $P < 0.01$).

between the latter two is only slight). *Synechococcus* constitutes the most original case because it displays more variability than the others, both in culture and in the field. This organism divides earlier during the day in coastal waters (e.g. off Woods Hole or in the Chesapeake Bay), which matches well with cultures, because strains without PE or with a low PUB:PEB ratio are characteristics of coastal waters (Olson et al. 1990, Wood et al. 1998), although the PUB:PEB ratio is probably a polyphyletic trait (Toledo et al. 1999). In contrast, late division, that is, after dusk, has been observed in the Mediterranean Sea. In the latter case, however, nutrient limitation may play a key role. Vaulot et al. (1996) showed that in the Mediterranean Sea, P depletion induced a clear retardation of DNA replication and therefore of cell division. Two other factors that also play a role for field surface populations are photoinhibition and excess UV radiation, both probably causing a delay in DNA replication and cell division (e.g. Jacquet et al. 1998a). Genetic variability may explain some differences between field and culture behavior, especially for eukaryotes for which cultured strains probably represent only a very small fraction of oceanic species (e.g. Moon-van der Staay et al. 2000).

Our study finally establishes that division rate can be roughly estimated from the ratio of the maximum to minimum forward scatter for a wide range of picoplanktonic species (Fig. 5), as demonstrated earlier for specific populations (e.g. Binder et al. 1996, Vaulot and Marie 1999). Such estimates should be useful because, for many field populations, scatter is easy to measure by FCM, whereas cell cycle analysis can be impossible due to either low cell concentration, interference with bacteria, or poor dye penetration into cells.

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TABLE 4. Timing of division cycle for *Prochlorococcus* under LD in culture and in the field.

Author(s)	Strain/population	LD	Timing of division
Culture studies			
Shalapyonok et al. (1998)		13:11 h	
Jacquet et al. (this study)	SS 120	12:12 h	
Jacquet et al. (this study)	PCC 9511	12:12 h	
Jacquet et al. (this study)	MED 4	12:12 h	
Field studies			
Liu et al. (1999)	Cent. Equat. Pacific		
Vaulot et al. (1995)	Equat. Pacific		
Blanchot et al. (1997)	Equat. Pacific		
André et al. (1999)	Equat. Pacific		
Liu et al. (1997)	Sub. Trop. N Pacific		
Campbell et al. (1998)	Arabian Sea		
Liu et al. (1998)	Arabian Sea		
Vaulot and Marie (1999)	Equat. Pacific		
Shalapyonok et al. (1998)	Arabian Sea		
Binder et al. (1996)	Cent. Equat. Pacific		
Partensky et al. (1996)	Trop. N E Atlantic		
Zubkov et al. (2000)	Atlantic ocean		
Jacquet et al. (this study)	Alboran Sea		

As photoperiod varies with locations and culture conditions, the period of division is represented by dark symbols (▬) and located approximately over a daily 24 h period without time precision but symbolized by dark and white bands (■□■) for night and day, respectively.

TABLE 5. Timing of division cycle for *Synechococcus* under LD in culture and in the field.

Author(s)	Strain	Phenotype	LD	Timing of division
Culture studies				
Mitsui et al. (1986)	BG43511/12	n. a.	12:12 h	
Armbrust et al. (1989)	WH 8101	No PE	14:10 h	
Binder and Chisholm (1995)	WH 8101	No PE	14:10 h	
Binder and Chisholm (1995)	WH 7803	Low PUB/PEB	14:10 h	
Waterbury et al. (1986)	WH 7803	Low PUB/PEB	14:10 h	
Mori et al. (1996)	PCC 7942	No PE	12:12 h	
Jacquet et al. (this study)	ROS 04	Low PUB/PEB	12:12 h	
Jacquet et al. (this study)	ALMO 03	Low PUB/PEB	12:12 h	
Jacquet et al. (this study)	WH 7803	Low PUB/PEB	12:12 h	
Campbell and Carpenter (1986)	WH 7803	Low PUB/PEB	14:10 h	
Campbell and Carpenter (1986)	WH 8107	High PUB/PEB	14:10 h	
Stramski et al. (1995)	WH 8103	High PUB/PEB	12:12 h	
Sweeney and Borgese (1989)	WH 7803	Low PUB/PEB	12:12 h	
Campbell and Carpenter (1986)	WH 8012	Low PUB/PEB	14:10 h	
Jacquet et al. (this study)	WH 8103	High PUB/PEB	12:12 h	
Field studies				
Waterbury et al. (1986)	Vineyard Sound			
Affronti and Marshall (1994)	Chesapeake Bay			
Carpenter and Campbell (1988)	Long Island Sound			
Li and Dickie (1991)	N Atlantic			
Sherry (1995)	NW Arabian Sea			
Xiuren and Vaulot (1992)	English Channel			
Waterbury et al. (1986)	Sargasso Sea			
Liu et al. (1998)	Arabian Sea			
Agawin and Agusti (1997)	NW Mediterranean Sea			
Neuer (1992)	Sub. Arct. Pacific			
Olson et al. (1990)	N Atlantic, Sargasso Sea			
Olson et al. (1990)	Vineyard Sound			
Kudoh et al. (1990)	NW Pacific			
Vaulot and Marie (1999)	Equat. Pacific			
Wyman (1999)	NE Atlantic			
Jacquet et al. (unpub. data)	Alboran Sea			
Vaulot et al. (1996)	NW Mediterranean Sea			
Campbell and Carpenter (1986)	Sargasso Sea, Carribean Sea			
Prézelin et al. (1987)	NW Atlantic			
Dolan and Simek (1999)	NW Mediterranean Sea			
Jacquet et al. (1998a)	NW Mediterranean Sea			

Phenotype is indicated by the absence of phycoerythrin (PE) or by the ratio of the chromophores phycourobilin (PUB) to phycoerythrobilin (PEB). n.a.: non available. Legend as in Table 4.

TABLE 6. Timing of division for picoeukaryotes under LD cycle in culture and in the field.

Author(s)	Strain/population	LD	Timing of division
Culture studies			
Jacquet et al. (this study)	*Bathycoccus prasinos*	12:12 h	
Jacquet et al. (this study)	*Bolidomonas pacifica*	12:12 h	
Jacquet et al. (this study)	*Micromonas pusilla*	12:12 h	
Jacquet et al. (this study)	*Pelagomonas calceolata*	12:12 h	
Jacquet et al. (this study)	*Pycnococcus provasolii*	12:12 h	
DuRand and Olson (1998)	*Nanochloris* sp.	14:10 h	
Field studies			
Jacquet et al. (1998a)	NW Mediterranean Sea		
Jacquet et al. (unpub. data)	Alboran Sea		
Blanchot et al. (1997)	Equat. Pacific		
André et al. (1999)	Equat. Pacific		
DuRand and Olson (1996)	Equat. Pacific		
Landry et al. (1996)	Cent. Equat. Pacific		
Vaulot and Marie (1999)	Equat. Pacific		

Legend as in Table 4. Except for Blanchot et al. (1997), who focused on a single population of organisms (as estimated by flow cytometry), other studies referred to the whole picoeukaryotic community.

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