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Effect of Phosphorus on the *Synechococcus* Cell Cycle in Surface Mediterranean Waters during Summer

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The effect of phosphorus (P) and nitrogen (N) additions on the *Synechococcus* cell cycle was tested with natural populations from the Mediterranean Sea in summer. In the absence of stimulation, the *Synechococcus* cell cycle was synchronized to the light-dark cycle. DNA synthesis began around 1600, a maximum of S-phase cells was observed at around dusk (2100), and a maximum of G_2-phase cells was observed at around 2400. Addition of P (as PO_4^{3-}) caused, in all cases, a decrease in the fraction of cells in G_2 at around 1800, no change at around 2400, and an increase at around 1200 the next day, while addition of N (as NO_3^-) had no effect. We hypothesize that P addition induced a shortening of the G_1 phase, resulting in cells entering and leaving the S and G_2 phases earlier. These data suggest very strongly that the *Synechococcus* cells were P limited rather than N limited during this period of the year. In most cases, additions as low as 20 nM P induced a cell cycle response. From dose-response curves, we established that the P concentration inducing a 50% change in the percentage of cells in G_2 was low, close to 10 nM, at the beginning of the sampling period (30 June) and increased to about 50 nM by the end (9 July), suggesting a decrease in the severity of P limitation. This study extends recent observations that oligotrophic systems may be P rather than N limited at certain times of the year.

The question of which nutrient(s) limits phytoplankton growth in oceanic waters is still very open. In the 1980s, the consensus was that nitrogen (N) was the key limiting nutrient in most marine systems, leading to the concepts of new and regenerated production, based on oxidized (nitrates) and reduced (ammonia) forms of nitrogen, respectively (17). However, this paradigm was shattered recently by the recognition that in some regions (HNLC [high nutrients, low chlorophyll] areas [14]), photosynthetic biomass remains low despite high nitrate levels. For some of these regions, such as the equatorial Pacific or the Antarctic, it was suggested, and then demonstrated, that the lack of iron (Fe), resulting from a low aerosol input, was limiting phytoplankton biomass (29, 30). Another nutrient that has attracted recent attention is phosphorus (P). Some regions subjected to freshwater input, such as the Chesapeake Bay, may shift from N to P limitation between summer and winter (20). Heterotrophic bacteria may also be P limited in these areas (50). The Mediterranean Sea is an area for which the idea of P limitation has been repeatedly invoked (3) because seawater exhibits an N/P ratio (31) larger than the intracellular Redfield ratio of 16:1 found in phytoplankton, possibly because of the removal of P by adsorption on Fe-rich dust particles (26). However, it is not clear whether P is limiting phytoplankton growth everywhere and at all times in the Mediterranean Sea. For example, N is probably limiting in winter in the western basin (36).

One reason for the continuing debate on what nutrient limits photosynthetic biomass in oceanic systems is the lack of adequate methods to demonstrate that a given nutrient is limiting. The comparison between the in situ dissolved N/P ratio and the Redfield ratio may suggest the nature of the limiting nutrient over broad temporal and spatial scales (26, 31). However, at shorter scales, imbalances between these two ratios may exist, and direct methods are necessary. These methods usually rely on bioassays performed by adding nutrients suspected to be limiting to incubation bottles (or even to open ocean areas, as recently demonstrated in the Equatorial Pacific by the IRONEX experiment [29]) and monitoring biological parameters over time. The key problem lies in the choice of the relevant parameters. In many cases, photosynthetic biomass (as chlorophyll) or production of carbon (^{14}C method) is used (16). However these variables respond very slowly to stimulation, usually after several days, when the community has dramatically altered its composition (e.g., shifting from picoplankton to diatoms) (12). Moreover, they reflect the net response of the community (i.e., the sum of gains and losses) and not the physiological responses of primary producers. This led to strong debates about the validity of the conclusions drawn from such experiments (2, 28). It is therefore highly desirable to monitor biological variables that respond quickly, such as biophysical parameters linked to photosynthesis, which can be evaluated by sophisticated fluorescence measurements (24).

Cell cycle variables are also very appropriate to monitor nutrient bioassays. In culture, phytoplankton cells that have been deprived of an essential nutrient usually arrest at the beginning of their cell cycle (G_1 phase) and then initiate DNA synthesis (S phase) within a few hours of being stimulated with a fresh addition (48). Similar behavior has also been observed for oceanic populations (49). The cell cycle status of natural populations can be easily determined by flow cytometry (6, 47, 49). This is especially convenient, since it allows one to monitor separately the response of a given autotrophic population (e.g., *Prochlorococcus* or *Synechococcus* organisms or the picoeucaryotes). This should provide more clear-cut responses than those observed by monitoring the whole community together (e.g., by total chlorophyll), since each population may have a different response (some negative and some positive) such that the average response may be vague.

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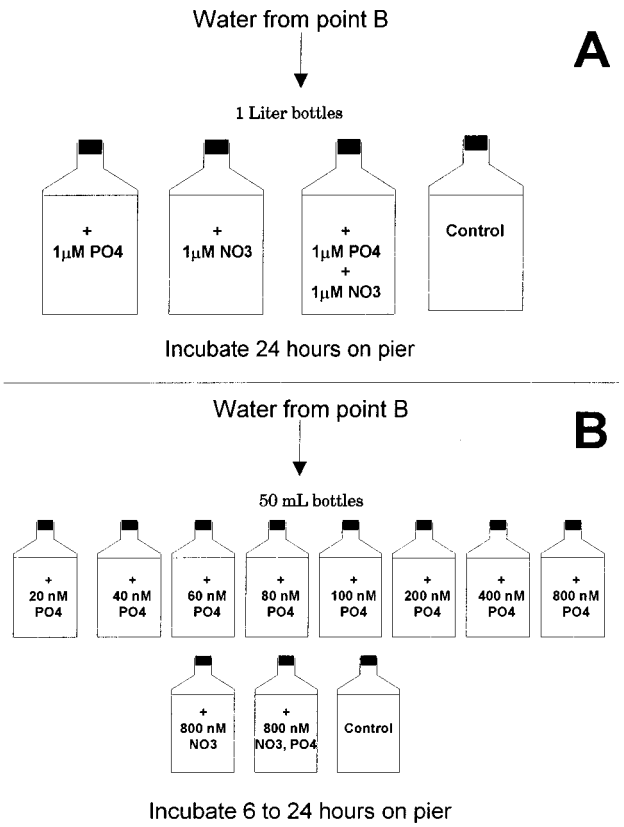

FIG. 1. Experimental design for nutrient additions. (A) Preliminary experiments. (B) Detailed experiments.

In the present study, we used the cell cycle status of natural *Synechococcus* populations to determine whether they are limited by P in the western Mediterranean Sea in summer.

MATERIALS AND METHODS

Study site. Water was sampled at the surface from point B about 500 m offshore at the entrance of Villefranche Bay (43°41′N, 7°19′E; depth, 80 m), a station located in the northwestern part of the Mediterranean Sea and very well characterized hydrologically (7).

Diel cycle analysis. Water was sampled at point B roughly every 3 h between 30 June 1993 at 1200 and 1 July 1993 at 1500. At the beginning of the diel cycle, a 20-liter carboy was filled with surface water, brought back to shore, and incubated on the pier of the Station Zoologique in running seawater with a 30% neutral density screen. Samples were then taken from the carboy with the same frequency as at point B or more frequently (every 1.5 h) during late evening and early night.

Nutrient enrichment type A. For a preliminary assessment of the limiting nutrient (Fig. 1A), water sampled at point B around midday was aliquoted into 1-liter polycarbonate bottles that received the addition of either PO_4-P (1 μM), NO_3-N (1 μM), or both PO_4-P and NO_3-N (1 μM) or received no addition (control), and the bottles were incubated as described above. Samples were taken at time zero and at about 24 h. This experiment was repeated twice (Table 1).

Nutrient enrichment type B. For a more detailed study of the effects of P concentration and of incubation time (Fig. 1B), water from point B was incubated in 50-ml culture flasks (Nunclon, Roskilde, Denmark) after the addition of either PO_4-P (in a range of concentrations from 20 to 800 nM), NO_3-N (800 nM), or both PO_4-P and NO_3-N (800 nM) or with no addition as a control. Samples were taken at time zero and after different incubation times (6, 12, and 24 h). This experiment was repeated 11 times between 30 June and 9 July 1993 (Table 1).

Flow cytometric analysis of *Synechococcus* cell cycle. Samples were fixed with 0.1% glutaraldehyde and frozen in liquid nitrogen (46). Just prior to analysis, samples were thawed and incubated with Hoechst 33342 (Sigma), a DNA-fluorescent stain, at 50 μg ml^{-1} and RNase at 0.1% (wt/vol) (final concentration) at 37°C for at least 1 h. This is a slight modification of the method of Monger and Landry (34), which does not include incubation at 37°C with RNase. RNase was used because in its absence *Synechococcus* cells were sometimes poorly stained and displayed a large coefficient of variation (CV) for the G_1 peak of the DNA distribution. RNase had two beneficial effects, as demonstrated for two marine *Synechococcus* strains, MAX01 and WH7803 (Fig. 2). First, the fluorescence of the G_1 peak was slightly higher and more stable. Second, the CV for the G_1 peak was improved by about 1%. Although the improvement was small, this consistently improved CV is very helpful for obtaining better estimates of the different

TABLE 1. Summary of all experiments performed[a]

Expt code	Incubation start		Incubation end		Effect[b] of:			Fitted parameters			r^2
	Date[c]	Time	Date[c]	Time	N	P	N + P	$G2_0$ (%)	ΔG2 (%)	P_{50} (nM)	
A01	30 June	1200	1 July	1200	=	+	+	NA[d]	NA	NA	
A02	1 July	1500	2 July	1200	=	+	+	NA	NA	NA	
B01	30 June	1200	30 June	1759	=	−	−	20.1	−11.8	16.5	0.98
B02	30 June	1800	30 June	2355	=	=	=				
B03	30 June	2400	1 July	0600	ND	=	=				
B04	1 July	0600	1 July	1205	=	=	ND				
B05	1 July	1200	1 July	1800	=	−	−	22.0	−13.7	13.3	0.95
			1 July	2359	=	+	ND	8.4	2.6	6.3	0.72
			2 July	1210	ND	+	ND	6.1	12.3	8.9	0.85
B06	1 July	1500	1 July	2359	=	=	=				
			2 July	1015	=	+	=	8.6	4.8	4.5	0.73
			2 July	1540	=	ND	+				
B07	2 July	1500	3 July	1510	=	=	=				
B11	6 July	1200	6 July	1810	=	−	−	37.0	−23.0	77.9	0.97
B12	7 July	1200	7 July	1824	=	=	ND				
			8 July	1025	ND	+	+	4.1	14.4	54.1	0.89
B13	8 July	1100	9 July	1130	=	+	+	0.0	21.4	55.0	0.85
B14	9 July	1600	9 July	2150	=	−	−	23.9	−12.9	36.5	0.81
			10 July	1530	=	=	=				

[a] Water sampled from point B off Villefranche-sur-Mer was incubated for various times after P and/or N addition (Fig. 1). Experiments that were started at the same time were given the same code. At the end of the incubation, the effect on the fraction of *Synechococcus* cells in G_2 was recorded. When an effect of P addition was observed, a model curve was fit with three parameters (equation 1). The r^2 value provides an idea of the goodness of fit.

[b] Effect on fraction of cells in G_2. =, no effect; −, decrease; +, increase; ND, not determined.

[c] All dates are in 1993.

[d] NA, not applicable.

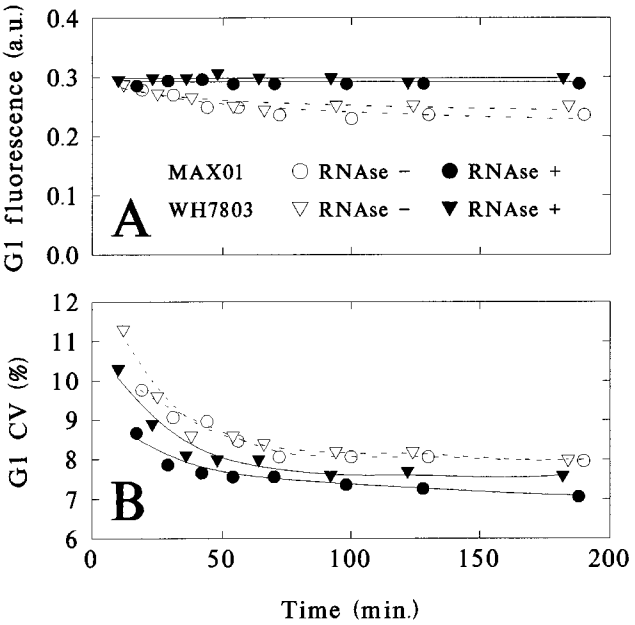

FIG. 2. Effect of RNase on DNA staining of *Synechococcus* cells with Hoechst 33342. (A) Intensity of the fluorescence of the G_1 peak related to that of 0.95-μm-diameter beads. (B) CV of the G_1 peak. Circles, strain MAX01; triangles, strain WH7803; open symbols, incubations at room temperature without RNase; closed symbols, incubation at 37°C with RNase.

cell cycle phase fractions. Moreover, with some difficult samples (not shown) the CV was improved by RNase addition much more than for the samples presented in Fig. 2. The positive effect of RNase could be due to the degradation of either rRNA or mRNA. The former may nonspecifically bind some of the Hoechst 33342 stain, and the latter, when still attached to DNA, may prevent an optimal accessibility of the stain.

Samples were then analyzed on an EPICS 541 flow cytometer as described previously (6). *Synechococcus* cells were discriminated on the basis of their right-angle scatter (a parameter related to size) and chlorophyll fluorescence (Fig. 3, top). The corresponding DNA histogram was recorded (Fig. 3, bottom) by using the CYTOPC software (44), and the percentages of cells in the cell cycle phases G_1, S, and G_2 were computed by using MCYCLE (Phoenix Flow Systems, San Diego, Calif.).

RESULTS

***Synechococcus* cell cycle in the Mediterranean Sea.** During the period of study (late June to early July 1993), *Synechococcus* cyanobacteria (Fig. 3) were the most abundant population of the phytoplankton at point B off Villefranche-sur-Mer, with typical concentrations of around 20,000 cells ml^{-1}. *Prochlorococcus* organisms probably were present, since divinyl-chlorophyll *a*, their marker pigment, can be measured in the water column at that time of the year (7), but they could not be detected, probably because of their very weak fluorescence. The concentration of photosynthetic picoeucaryotes was around 1,000 cells ml^{-1}. Because of their abundance, *Synechococcus* bacteria therefore constituted the most appropriate population for cell cycle analysis. By improving a previously used DNA staining protocol (see Materials and Methods), we obtained well-resolved DNA distributions with either one or two peaks (Fig. 3), which we call G_1 and G_2 by analogy to the eucaryotic cell cycle phases (49), separated by cells in the process of replicating DNA (S phase). The CV of the G_1 peak was on average 15%, allowing for a correct separation of the cell cycle phases.

Our first step was to establish the phasing of the *Synechococcus* cycle in situ and to determine whether it was disturbed when water was incubated under simulated in situ conditions. The *Synechococcus* cell cycle was clearly synchronized (Fig. 4). S phase began at around 1600 with a sharp maximum at 2100 (close to sunset). The fraction of cells in G_2 began to increase around that time and declined at dawn the next morning. Using the model of Carpenter and Chang (9), we estimated a division rate of 0.73 day^{-1}, that is, slightly in excess of one doubling per day. Water incubated at 30% of the surface light intensity also exhibited a synchronized cycle, although the percentage of cells in S phase peaked 3 h earlier than it did in situ because of an advanced initiation of DNA replication (Fig. 4).

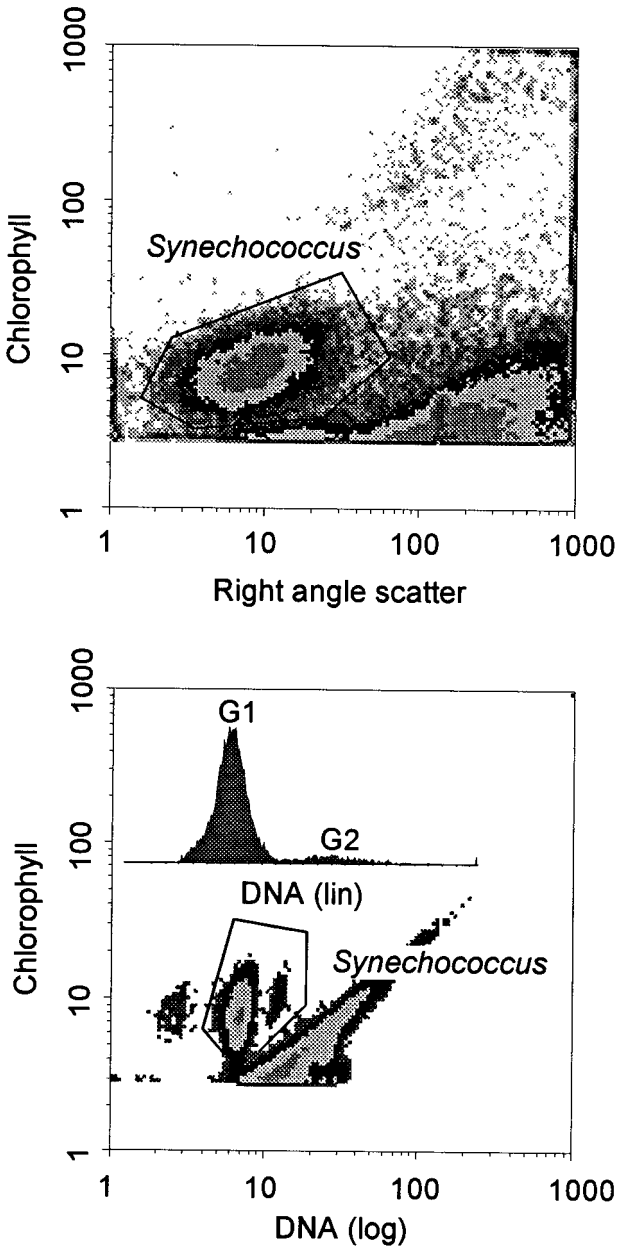

FIG. 3. Flow cytometric analysis of natural *Synechococcus* populations from surface waters in Villefranche Bay (1 July 1993, 0300), stained with Hoechst 33342 after incubation with RNase. (Upper panel) Right-angle scatter (a function of cell size) versus chlorophyll fluorescence. (Lower panel) DNA versus chlorophyll fluorescence. The inset shows the *Synechococcus* DNA distribution on a linear scale, with a major peak in G_1 and a minor peak in G_2.

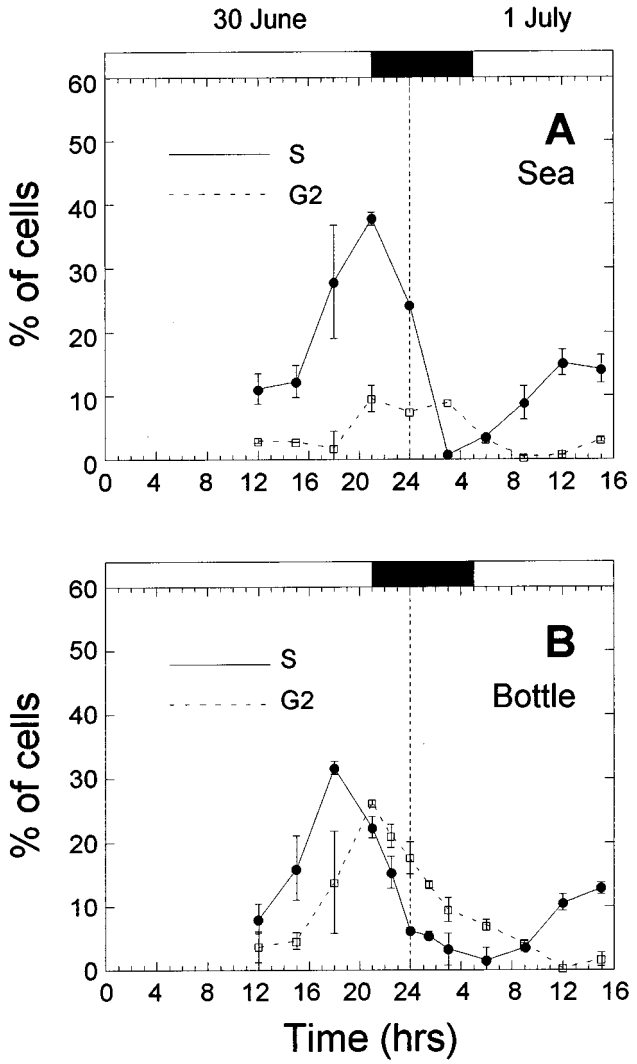

FIG. 4. Diel variability of the percentage of *Synechococcus* cells in the S (●) and G_2 (□) phases of the cell cycle. (A) Surface samples from point B off Villefranche-sur-Mer. (B) Samples from a bottle filled with water taken from point B at 1200 on 30 June 1993 and incubated at 30% of the surface light intensity in a circulating seawater bath. The dashed line corresponds to midnight, and the black box corresponds to night hours. Error bars correspond to standard deviations for two independent cell cycle analyses, except in panel A for the first two datum points of 1 July, for which only one determination was made.

Moreover, the maximum percentages of cells in the S and G_2 phases were, respectively, slightly higher and lower than they were in situ. In fact, these changes compensated each other, such that the estimated division rate in the bottle (0.78 day^{-1}) was nearly identical to that in situ.

Effect of nutrient addition on *Synechococcus* cell cycle. In order to test the effect of nutrient addition on the *Synechococcus* cell cycle, we designed two types of experiments (Fig. 1). The initial (A) experiments consisted of incubating 1-liter bottles with four types of additions during 24 h. Cell cycle distributions were obtained by flow cytometry at both the beginning and the end of the incubation. On both occasions when A experiments were performed (Fig. 5; Table 1), they clearly demonstrated that populations originating from a midday sample and incubated with either P or both P and N exhibited, after 24 h, an increased proportion of cells in G_2 in comparison with a control or with cells receiving only N. These initial experiments raised several questions. First, how does the cell cycle response vary with the concentration of P added? Second, is the response always the same, no matter when incubation starts or stops?

To address these questions, we performed the B experiments, which included P addition in a range of concentrations as well as variations in both the start time and incubation duration (Fig. 1; Table 1). These experiments confirmed the A experiments. At 800 nM, N addition did not change the cell cycle compared with controls, while joint N and P additions had the same effect as P addition alone. Moreover, the effect on the cell cycle was clearly related to the added P concentration (Fig. 6). This effect could be adequately modeled by a hyperbolic function of the form:

$$G2 = G2_0 + \Delta G2 \cdot \left[1 - \exp\left(-\ln 2 \frac{P}{P_{50}}\right)\right] \qquad (1)$$

where $G2$ is the observed percentage of cells in G_2 at the end of the incubation and P is the added P concentration (in nanomolar). The fitted parameters were $G2_0$, $\Delta G2$, and P_{50}: $G2_0$ is the percentage of cells in G_2 in the absence of P addition, $\Delta G2$ is the change (positive or negative [see below]) as a result of saturating P addition, and P_{50} is the concentration that induced a 50% effect compared with the maximum effect. Equation 1, when it could be fitted (i.e., when a significant effect could be detected), accounted for a very high percentage of the variance in the data (Table 1).

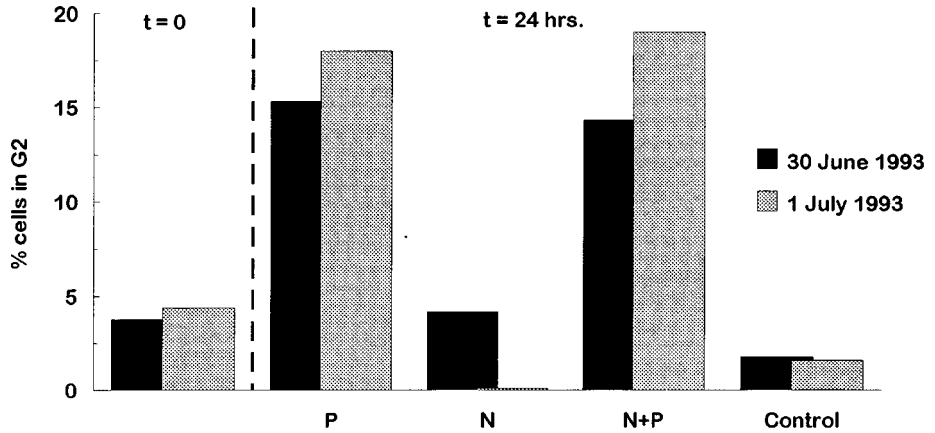

FIG. 5. Effect of nutrient additions on the percentage of *Synechococcus* G_2-phase cells in the A experiments. Surface samples were taken at around noon from point B off Villefranche-sur-Mer on 30 June and 1 July 1993, spiked with 1 μM PO_4-P, 1 μM NO_3-N, or both, and incubated for 24 h. Control, no addition.

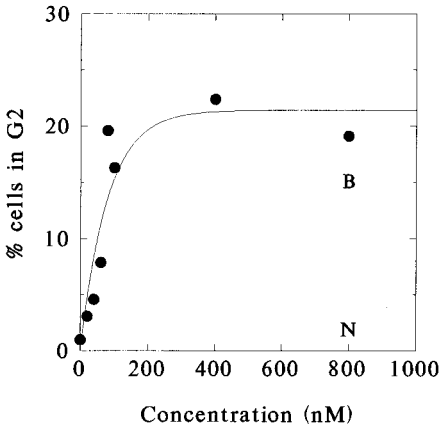

FIG. 6. Experiment B13 (Table 1), started on 8 July 1993 at 1100 and ended on 9 July 1993 at 1130. The effect of increased PO_4-P addition on the percentage of *Synechococcus* G_2-phase cells after 24 h of incubation is shown. N, addition of NO_3-N at 800 nM; B, addition of both PO_4-P and NO_3-N at 800 nM. The line corresponds to equation 1 ($\Delta G2 = 21\%$, $P_{50} = 55$ nM PO_4-P).

The observed effect was clearly dependent on the time of the day (Fig. 7), as expected from the phasing of the *Synechococcus* cell cycle (Fig. 4). We observed a negative effect on the percentage of cells in G_2 when the incubation was stopped in late afternoon on the same day it was started (Fig. 7A), no effect when the incubation was stopped at around midnight (Fig. 7B), and a positive effect when the incubation was stopped in early afternoon on the next day (Fig. 7C). This trend is confirmed when the entire data set is examined (Fig. 8A). Positive effects occurred around 1200, negative effects occurred between 1800 and 2200, and there was no effect for other ending times. In contrast, the start time did not seem to have any effect (Table 1). Finally, the concentration that induced a 50% effect (P_{50}) was not constant during the experimental period. Instead, it was very low (5 to 20 nM) in the first days of July, increased to 80 nM around 6 July, and then decreased slowly thereafter (Fig. 8B).

DISCUSSION

***Synechococcus* cell cycle phasing.** Numerous studies have revealed that in culture, phytoplankton division and the cell cycle are closely regulated by light-dark cycles (13, 18). Although the exact mechanisms that induce cells to synchronize to light-dark cycles are still unknown, mounting evidence demonstrates that a large number of cellular processes, as well as many genes, express circadian rhythmicity, not only in eucaryotes (33) but also in procaryotes (25, 27). However, very little is known about the phasing of the cell cycle of phytoplankton in marine waters. Early studies based on microscopic observations revealed, for example, that dinoflagellate division was phased to the diel cycle and took place at night (41). For *Synechococcus* cells, the percentage of paired cells peaked at around sunset in the Sargasso Sea (8), suggesting that the cell cycle of this procaryote is also phased in situ. However, high-quality data on the cell cycle of natural populations have been obtained only recently with the use of DNA-fluorescent stains, such as DAPI (4′,6-diamidino-2-phenylindole) or Hoechst 33342 (10, 11). In particular, this technique revealed that the cell cycle of the *Prochlorococcus* photosynthetic procaryote is highly synchronized to the diel cycle in both the equatorial Pacific and the tropical Atlantic (38, 47). The present data set demonstrates that the *Synechococcus* cell cycle is also highly synchronized to the diel cycle in surface Mediterranean waters. As is the case for surface *Prochlorococcus* populations at the equator (47), the occurrence of the maximum number of cells in the S phase coincided with sunset (Fig. 4A). It should be noted that sunset occurs much later in the Mediterranean Sea in summer (2100) than at the equator (1730). The *Synechococcus* synchrony was not as tight as the *Prochlorococcus* synchrony, however, since some cells had already begun to enter the S phase in late morning (Fig. 4A). When a field sample was incubated in a bottle receiving 30% of the surface solar irradiance, cells were still synchronized (Fig. 4B). However, the peak of the S phase occurred about 3 h earlier (1800). Interestingly, the S phase of natural *Prochlorococcus* populations is also triggered earlier at 30 m (where cells receive about 20% of the surface light) than at the surface, suggesting that elevated light levels may cause a delay in DNA synthesis, possibly as a protective mechanism against UV damage (47). The high degree of synchrony observed for *Synechococcus* organisms resulted in a division rate slightly greater than once per day, which is in agreement with previous estimates of *Synechococcus* growth in these waters (19).

In cultured marine *Synechococcus* strains, two types of cell cycle distributions have been observed (1, 4, 5). One type displays more than two peaks, which have DNA contents corresponding to 1, 2, and 3, etc., genome copies (e.g., for marine strain WH7803); this is called the asynchrony phenotype (4), because it is suggestive of an asynchronous initiation of replication forks, in contrast to the classical model of bacterial chromosome replication (21). Clearly, such a phenotype was

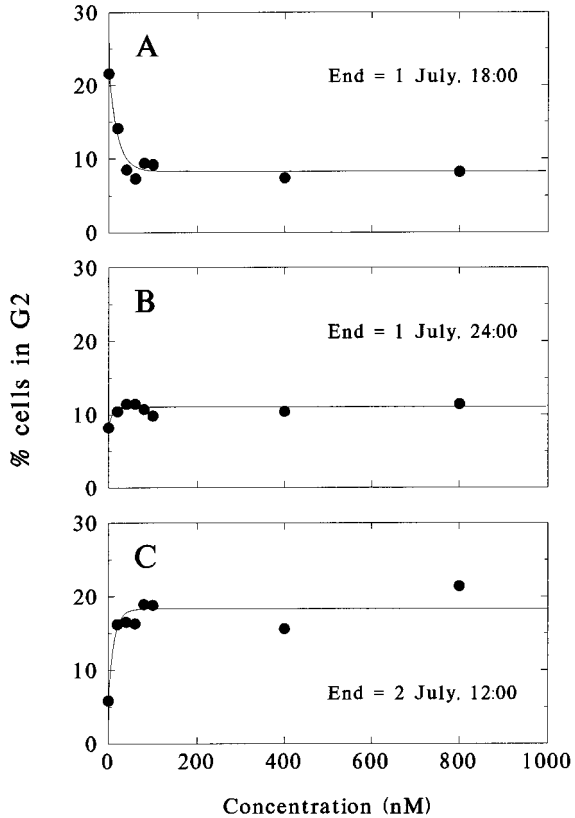

FIG. 7. Experiment B05 (Table 1), started on 1 July 1993 at 1200. The effect of incubation end time on the percentage of *Synechococcus* cells in the G_2 phase of the cell cycle is shown. The lines correspond to equation 1 (see Table 1 for the values of the fitted parameters).

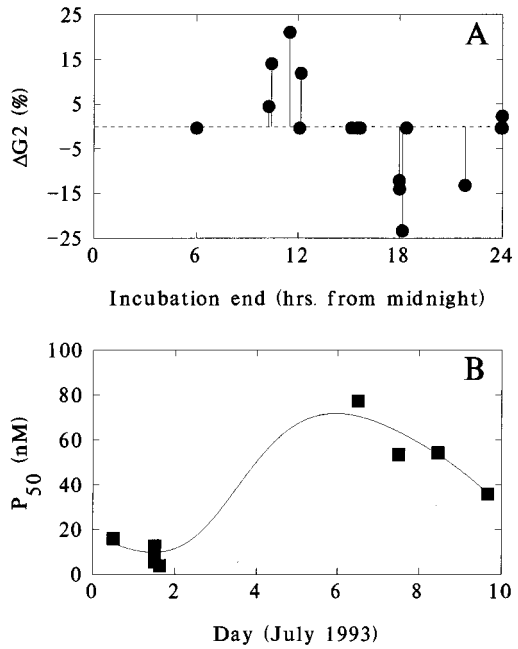

FIG. 8. B experiments. (A) Relation between the maximum change in G_2 (ΔG2 [equation 1]) and the time at which the incubation ends (in hours from midnight). (B). Evolution of the P concentration inducing a 50% effect on the cell cycle (P_{50} [equation 1]) during early July 1993. The solid line corresponds to a spline fit of the data.

not observed in natural Mediterranean populations. The other type displays a bimodal cell cycle (e.g., for strain WH8103) matching the normal eucaryotic phenotype and corresponds to what was observed during this study. In cultured strains, however, cells are poorly phased and a significant part of the population always remains in S or G_2 during darkness, such that the G_1 fraction never reaches 100% (1, 5). In natural Mediterranean populations, synchrony was tighter, with the G_1 fraction reaching up to 100% (Fig. 4). The better synchrony under field conditions might be due to the temporal modulation of natural sunlight. In cultures, light is usually turned on and off abruptly, while in nature it gradually increases and decreases in a sine wave-like fashion. For example, the near-perfect synchrony observed in nature for the closely related *Prochlorococcus* procaryote (47) cannot be reproduced in light-on, light-off cultures (45a).

Effect of P addition on the *Synechococcus* cell cycle. Our nutrient enrichment experiments were performed under simulated in situ conditions, which induced the S phase to occur earlier than it does under natural conditions (see above). However, since all bottles were subjected to similar conditions, different treatments can be unambiguously compared. In all cases, cell cycle distributions were not different in N-supplemented and control bottles. In contrast, additions of P as well N plus P always had identical effects, which could be either positive, negative, or null in terms of the percentage of cells in G_2. The direction of the effect was clearly dependent on the time at which the incubation was ended (Fig. 7 and 8A). The most parsimonious explanation of this effect is that P addition caused an advance in the last part of the *Synechococcus* cell cycle, such that the G_2 phase took place around midday rather than at sunset. This hypothesis accounts for the fact that the relative change in G_2 (ΔG2) was always positive around noon and negative around dusk. The advance in G_2 timing was most likely due to the shortening of the G_1 phase in response to P addition. Although S may also have been shortened, the observed advance is too large (8 to 10 h) to be accounted for solely by a reduction in the duration of S, which lasts 2.7 and 4.5 h under incubation and in situ conditions, respectively, as computed according to the model of Carpenter and Chang (9) from the data of Fig. 4. Since we did not monitor a full diel cycle after P addition, we do not know whether P addition simply shifted the whole cell cycle with respect to the diel cycle or if it allowed a second wave of cells to replicate their DNA in the late afternoon and to divide at night, increasing the population growth rate much beyond one division per day. The fact that P addition shortens the duration of G_1 (and, conversely, that P limitation increases it) agrees with what is known about marine phytoplankton cell cycle control by environmental factors (45). For example, both nitrogen and light limitations usually induce cells to spend more time in G_1 (35). The effect of phosphorus has been much less studied. A recent study of *Prochlorococcus* spp. demonstrates, however, that P starvation affects not only the G_1 phase but also both S and G_2 (37).

Ecological consequences. Although it is close to shore, point B off Villefranche-sur-Mer represents typical Mediterranean Sea oligotrophic conditions in summer, with a permanent thermocline and nitrate levels below the detection limit of classical methods (100 nM), except for short periods when strong wind-induced mixing takes place (7). P concentrations are also very low in summer (42). The present data clearly demonstrate that the cell cycle of cells of the *Synechococcus* genus, an important photosynthetic procaryote, is controlled by phosphorus and not by nitrogen in summer. It is likely that the *Synechococcus* division rate was also increased following P addition, despite its already high value (close to one doubling per day) under the natural P-limited conditions (Fig. 4). Other evidence demonstrates that, during the same period, bacteria were also physiologically P limited at this site (43), suggesting that phosphorus may control both auto- and heterotrophic populations simultaneously. Estimated values for the biologically available P (between 1 and 30 nM, depending on methods and assumptions [43]) coincide with the range of concentrations that accelerated the *Synechococcus* cell cycle, as represented by P_{50} in equation 1 (Fig. 8B). These very low values also agree with those known to limit the *Prochlorococcus* growth rate (30 nM [37]) or to induce *Synechococcus* production of the phosphate-binding protein PhoS (40) (below 50 nM [39]).

The increase in the P_{50} concentration between 1 July and 7 July may have corresponded to a brief relaxation of the severity of P limitation during that period as a result of an increase in the P concentration in the mixed layer. Indeed, the meteorological record shows a slight increase in average wind speed from 3 m s^{-1} on 1 July to 5.4 m s^{-1} on 4 July, which may have been sufficient to inject phosphorus from below the thermocline (23). An increase in P turnover time was indeed observed during the same period (43). After July 7, the P_{50} concentration dropped again, probably in response to increased P limitation, following the consumption of mixed-layer P by both auto- and heterotrophs.

Our data reinforce a growing body of evidence that P limitation might be much more widespread in oligotrophic environments than previously thought. For example, the tropical Pacific may shift from N to P limitation during El Niño years (22). In the Sargasso Sea, the N/P ratio is usually in excess of the Redfield ratio (32), and recent evidence points to bacteria being P limited (15). The shift from N- to P-limited marine ecosystems has important consequences for our conceptualization of biogeochemical fluxes, since these two elements have different remineralization pathways and rates (42). Examina-

tion of the cell cycle responses of specific autotrophic (and possibly heterotrophic) populations to nutrient additions constitutes a powerful tool to assess limiting nutrients in oceanic waters.

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