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RAPID RESPONSE PAPER

Cell cycle distributions of prochlorophytes in the north western Mediterranean Sea

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Abstract—Natural populations of oceanic prochlorophytes were sampled from the northwestern Mediterranean Sea in winter, stained with the DNA-specific fluorochrome DAPI, and analysed by flow cytometry. DNA histograms exhibited two peaks (G_1 and G_2 cells, containing one and two genome copies, respectively), separated by a trough of DNA-synthesizing cells (S cells). This suggested a cell cycle with a discrete S phase similar to that observed in eucaryotes or slow-growing procaryotes. Nitrogen and light were the two key environmental factors controlling the *in situ* cell cycle distributions of this procaryote. When nitrate levels were below 0.4 μM or light below 0.1% of the surface intensity, most of the cells were found in G_1, suggesting that they were not actively cycling. Cells arrested in G_1 could be induced to cycle into S + G_2 by incubating them with added nitrogen. Response was a function of initial nitrate concentration and decreased with depth, indicating that it was modulated by available light. These findings strongly suggest that prochlorophytes, which are one of the key components of the picoplankton community, may grow slowly in nitrogen-depleted waters, but still have the potential to respond quickly to nitrogen pulses.

INTRODUCTION

THE *in situ* growth rate of phytoplankton is one of the key quantities that need to be determined to reach a better understanding of oceanic production (FURNAS, 1990).

Most methods currently available are indirect, relying on biochemical markers. Typically, the incorporation of a labelled compound into essential cell components is measured and the growth rate is deduced after making a number of assumptions on elemental ratios and living matter composition (FURNAS, 1990). These methods have two main disadvantages. First, they involve incubations, that are known to be prone to artefacts such as sample confinement or cell poisoning (e.g. WILLIAMS and ROBERTSON, 1989). Second, they are global, i.e. they do not discriminate individual components (cell or population) of the phytoplankton community. Recent progress, such as the incorporation of labelled compounds into individual cells (RIVKIN and SELIGER, 1981) or into particular photosynthetic pigments (GIESKES and KRAAY, 1989), should lead to more accurate and specific measurements.

In contrast, direct methods rely on monitoring the population dynamics of phytoplankton. Ideally, the most straightforward way to estimate population growth rates is

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from time series of cell numbers. Because of diffusion and grazing, however, only net growth rates are obtained this way. To compute absolute growth rates, diffusion rates need to be measured (e.g. see LANDE *et al.*, 1989), while grazing must be either estimated (e.g. by the dilution method of LANDRY and HASSETT, 1982) or eliminated (e.g. by prescreening; FURNAS, 1991). An alternative method, that can be traced back to GOUGH (1905), is to determine the frequency of dividing cells, from which the growth rate can be computed, provided that data are collected at narrow intervals over the diel cycle and the duration of the division phase (t_d) is known (MCDUFF and CHISHOLM, 1982). Despite its potential advantages (direct measurement, no need for incubation), this method has been relatively little used because (1) it is tedious, (2) it is only applicable to species with easily identifiable division forms (e.g. dinoflagellates, diatoms, or *Synechococcus* cyanobacteria), and (3) the necessity to determine t_d is a major source of error and uncertainty (MCDUFF and CHISHOLM, 1982). The first drawback can be removed by using automated recording techniques such as image analysis or flow cytometry. The second and third problems are solved by obtaining more details on the population structure through the measurement of the DNA distribution and the estimate of the percentage of cells in each phase of the cell cycle: the initial growth phase G_1, the DNA synthesis phase S, and the late growth and mitosis phases G_2 + M. In this case, the length of the division phase (t_d) is replaced by the duration of S + G_2 + M, which can be determined directly from time series of field DNA distributions (CARPENTER and CHANG, 1988). However, before using this method to measure the growth rate of natural populations, it is necessary to obtain data on DNA distributions in the ocean.

We recently developed a method to measure phytoplankton cell DNA by flow cytometry in natural samples (BOUCHER *et al.*, 1991). In the present paper, we apply this method to oceanic prochlorophytes in the Mediterranean Sea. These very small (0.4–0.8 μm) photosynthetic procaryotes (CHISHOLM *et al.*, 1992) contain unique chlorophyll analogs (divinyl chlorophyll *a* and *b*, recently called chlorophyll a_1 and b_1, and a chlorophyll *c*-like pigment), GOERICKE and REPETA, in press) and are extremely abundant (10^5 cell ml^{-1}) in the Atlantic and Pacific Oceans (CHISHOLM *et al.*, 1988), as well as in the Mediterranean Sea (VAULOT *et al.*, 1990). The measured DNA distributions allow us to estimate the fraction of cells in S + G_2, an index that is analogous to the frequency of dividing cells (FDC), widely used for bacteria (NEWELL and CHRISTIAN, 1981). Our data suggest that both inorganic nitrogen and light are essential factors controlling *in situ* prochlorophyte cell cycle. This is corroborated by incubations of samples in the presence of added nitrogen and agrees with what is known about cell cycle control in photosynthetic populations.

METHODS

Sampling and hydrological parameters

From three to eight stations were occupied along each of three transects (ZA, ZC, ZD) originating from the Rhône river estuary and extending into the northwestern Mediterranean Sea (Fig. 1) between 29 December 1988 and 8 January 1989 during the second leg of cruise 179 of the R.R.S. *Discovery*. Three to 10 depths were sampled with 10-l Go-Flo bottles depending on bottom depth and on the shape of the density and fluorescence profiles measured with a CTD. Nutrients (NO_3^-, NH_4^+ and PO_4^{2-}) were determined

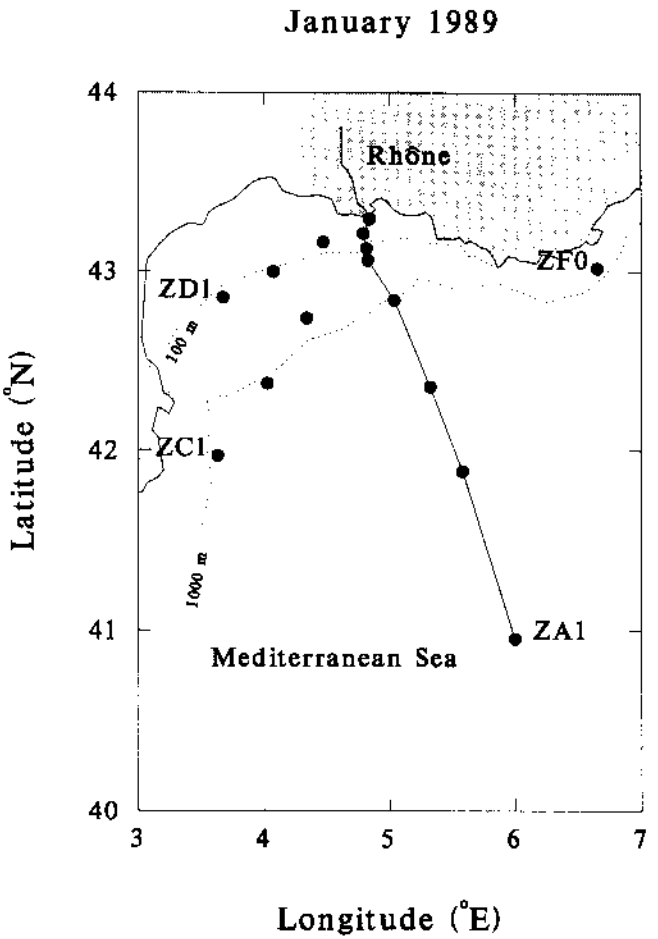

Fig. 1. The stations sampled in the northwestern Mediterranean Sea in December 1988–January 1989 were distributed (except for ZF0) along three transects (ZA, ZC and ZD) and numbered from offshore to inshore. Vertical cross-sections along transect ZA are presented in Fig. 3

on-board with a Technicon Autoanalyser (Woodward and Owens, 1989). Chlorophyll concentration was obtained by converting CTD fluorescence after calibration with HPLC Chl *a* measurements (Vaulot *et al.*, 1990)

Since no measurements of the underwater light field were undertaken during the cruise, we estimated, by iteration, euphotic zone depth (Z_{eu}) from euphotic zone chlorophyll content ($\langle C_{tot} \rangle$) using the statistical relation (Morel, 1988):

$$Z_{eu} = 568.2 \langle C_{tot} \rangle^{-0.746}.$$

In situ *incubations*

At four stations (ZA1, ZA4, ZA6 and ZC1), water was retrieved from 1 to 5 depths 1 h before dawn, put in four polycarbonate bottles, spiked with either $N\text{-}NO_3^-$ (0.1 or 1 μM) or $N\text{-}NH_4^+$ (0.1 or 1 μM), and incubated for 24 h (*in situ* during the daylight period and on

deck at night). The surface sample was usually split into two replicates incubated at 1 and 3 m. Samples at $t = 0$ and $t = 24$ h were obtained courtesy of N. J. P. Owens and A. P. Rees (Plymouth Marine Laboratory, U.K.).

Flow cytometry

Sample preservation, DNA staining and flow cytometry measurements were as described in BOUCHER *et al.* (1991). Briefly, samples were fixed with 1% glutaraldehyde and stored in liquid nitrogen (VAULOT *et al.*, 1989). Shortly before analysis, DAPI was added to the thawed sample at a final concentration of 2 μg ml^{-1}. Samples (300 μl) were analysed on an EPICS 541 (Coulter, Hialeah, FL, U.S.A.) flow cytometer using a high-sensitivity Biosense flow cell and 200 mW of UV light. DAPI-DNA blue fluorescence was collected at 485 ± 22 nm and chlorophyll red fluorescence was collected above 690 nm. Right angle light scatter and chlorophyll fluorescence were collected on three-decade logarithmic scales while DNA was collected both on logarithmic and linear scales. One micrometre Polysciences (Warrington, PA, U.S.A.) and 2-μm Pandex (Mundelein, IL, U.S.A.) beads, as well as calf thymus nuclei (Ortho Diagnostic Systems, Westwood, MA, U.S.A.), were used as fluorescence standards. Oceanic prochlorophytes were discriminated from picoeucaryotes by their much smaller scatter and red fluorescence, and from cyanobacteria by the absence of phycoerythrin orange fluorescence (VAULOT *et al.*, 1990). Their identity was confirmed by the presence of Chl a_1 (GOERICKE and REPETA, in press) in size-fractionated (GF/F-0.6 μm) samples where they dominated (VAULOT *et al.*, 1990). DNA histograms (Fig. 2) were obtained by gating the prochlorophyte population on both light scatter and chlorophyll fluorescence (BOUCHER *et al.*, 1991). Histograms with less than 500 recorded cells were discarded. DNA histograms displayed two peaks, corresponding to the G_1 and G_2 phases of the cell cycle, separated by a trough of S phase cells (see Discussion). Since the resolution of the peaks was not always sufficient to allow precise estimates of the different phases of the cell cycle (G_1, S, G_2), histograms were simply analysed by ascribing cells either to G_1 or S + G_2 using a fixed boundary at the base of the G_1 peak. We present the data as the fraction of cells in S + G_2 (f_{S+G_2}). The precision on replicate analyses was fairly high: for example at Sta. ZA1 in surface the coefficient of variation of f_{S+G_2} was equal to 8% for $n = 4$.

RESULTS

In situ *prochlorophyte cell cycle distributions*

During late December and early January, northwestern Mediterranean Sea waters were stratified offshore and mixed in the Golfe du Lion (continental shelf). Offshore surface waters were nitrate-depleted with a maximum nitracline depth of about 60 m. The major source of nutrients was the "chimney" located at about 42°N, 5°E (Fig. 3A). It is a typical winter-time feature of this zone, associated with deep water formation (GASCARD, 1978; CARAUX and AUSTIN, 1983). In the middle of the chimney, mixing brought NO_3^- concentrations up to 5 μM, while, outside the chimney, concentrations dropped rapidly with steeper gradients towards the coast than in the offshore direction (Fig. 3A). The influence of the Rhône river did not extend very far offshore at this period of the year: only

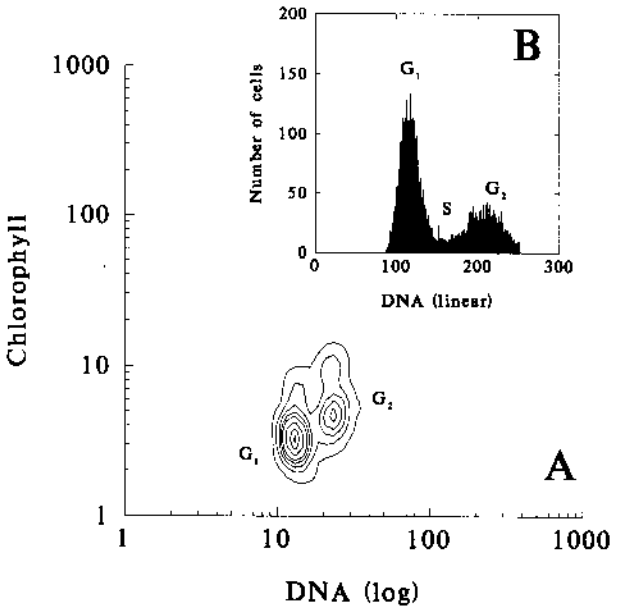

Fig. 2. Flow cytometric analysis of prochlorophyte DNA for a sample taken at 20 m-depth in the northwestern Mediterranean Sea in winter (Sta. ZA2, 41°53'N, 5°35'E, 31 December 1988, 1.05 a.m.). (A) DNA-DAPI fluorescence vs chlorophyll fluorescence (both expressed in arbitrary units on three-decade log scales). Prochlorophytes were gated on right-angle light scatter vs chlorophyll fluorescence cytograms (VAULOT *et al.*, 1990). (B) DNA fluorescence histogram (linear scale). Coefficient of variation of the first peak was 9.2%. Ratio of the fluorescence of the two peaks was 1.84. Ratio of the height of the first peak to the trough of the interpeak region ≅15. Phases are named in analogy with those of the eucaryotic cell cycle.

Sta. ZA6 exhibited a surface layer of lower salinity enriched in nutrients (VAULOT *et al.*, 1990).

The distribution of prochlorophytes was strongly influenced by the physical setting. In the chimney itself, concentrations were low and fairly homogenous vertically as a result of strong mixing (Fig. 3B). Away from the chimney, concentrations increased and vertical gradients became steeper; these tendencies were more pronounced in the offshore than in the inshore direction. At Stas ZA1 and ZA4, layers with high concentrations of prochlorophytes were observed at depth (80 and 200 m, respectively): they were associated most likely with cooled surface water rapidly sinking at the edge of the chimney (GASCARD, 1978; VAULOT and PARTENSKY, 1989). The proportion of cells in the S and G_2 cell cycle phases (f_{S+G_2}) presented a very interesting pattern (Fig. 3C): in surface waters, f_{S+G_2} was low (4%) at Stas ZA1 and ZA4, where nutrient concentrations were low, and high at Sta. ZA2 near the edge of the chimney and onshore of Sta. ZA5 in the direction of the Rhône river mouth in concordance with higher nutrient concentrations (Fig. 3). Vertically, f_{S+G_2} was fairly uniformly low throughout the water column at stations where it was low in surface waters (Fig. 4, ZA1). However, a slight maximum was observed repeatedly at the nitracline level (Fig. 4). At stations where f_{S+G_2} was high in surface waters (Fig. 4, ZA2), it decreased with depth.

These data suggest that both nutrients and light were important environmental factors

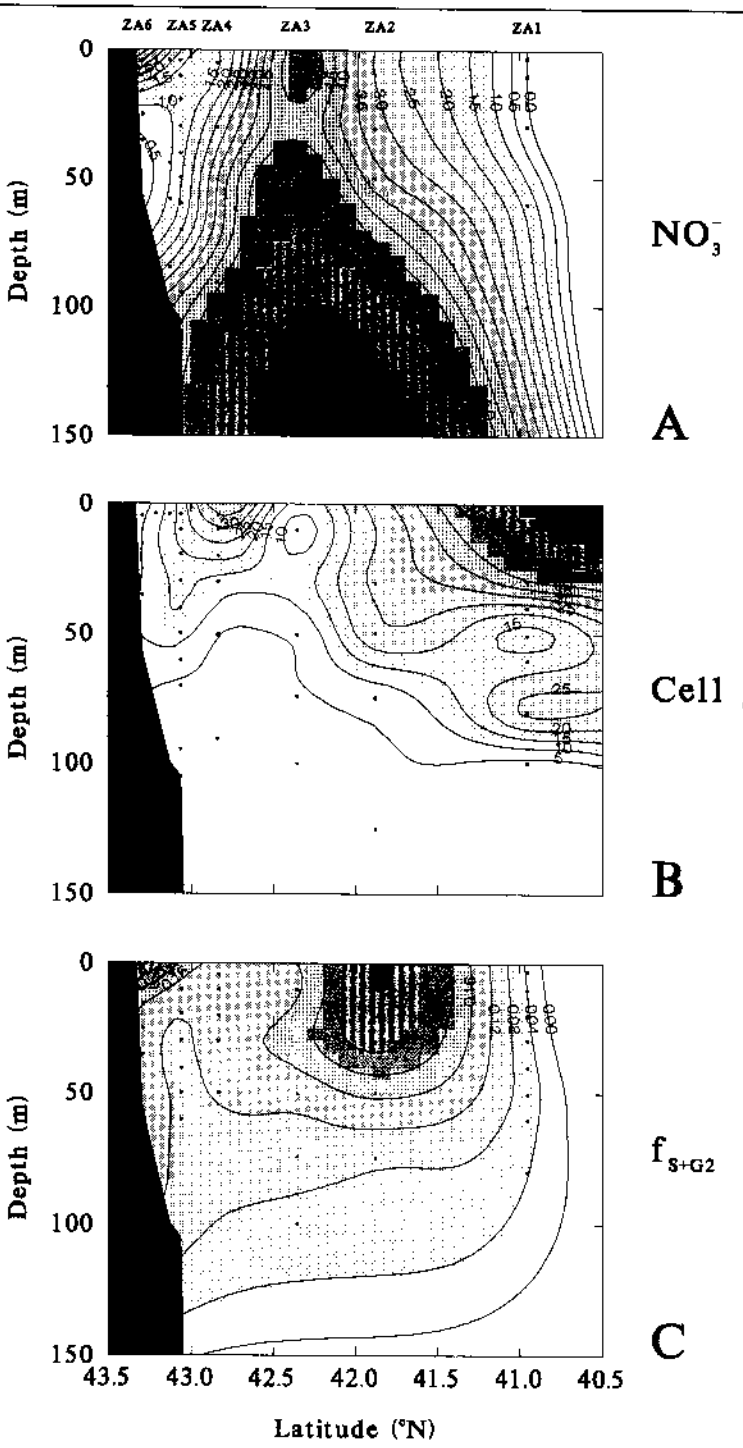

Fig. 3. The ZA transect. Dots indicate actual sample depths. (A) Nitrates (μM). (B) Prochlorophyte concentration (cell μl^{-1}). (C) Fraction of prochlorophyte cells in phases S and G_2 (f_{S+G_2}). Nutrient data were obtained courtesy of M. Woodward (Plymouth Marine Laboratory, U.K.).

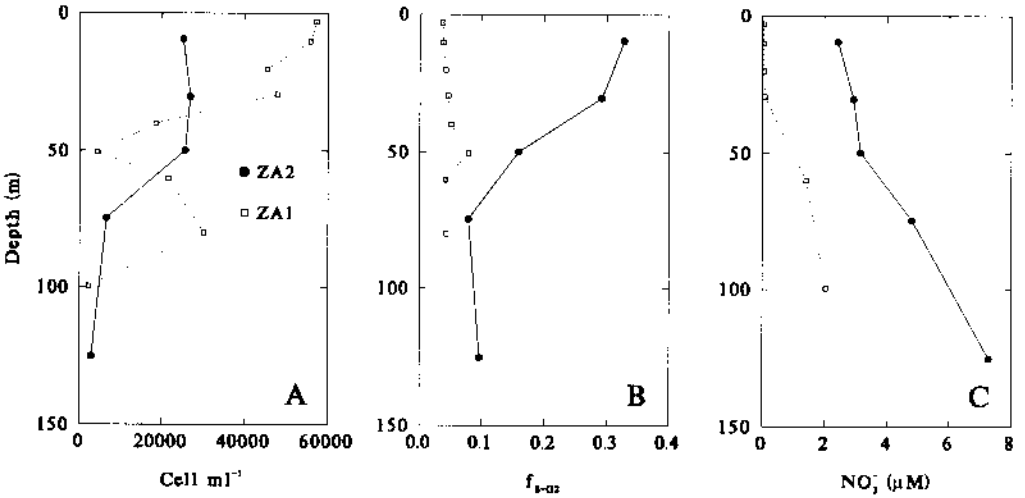

Fig. 4. Vertical profiles at two stations sampled in winter in the northwestern Mediterranean Sea (ZA1: 40°57′N, 6°00′E, 30 December 1988, 7.53 a.m.; ZA2: 41°53′N, 5°35′E, 31 December 1988, 0.15 a.m.). (A) Prochlorophyte concentration. (B) Fraction of cells in S + G_2. (C) Nitrates. Nutrient data were obtained courtesy of M. Woodward (Plymouth Marine Laboratory, U.K.).

that determined cell cycle distributions in the Mediterranean Sea. When f_{S+G_2} was plotted against NO_3^- concentration (Fig. 5A) for all samples, there was a clear threshold around 0.4 μM, below which no more than 5% of prochlorophyte cells were in S or G_2. For higher NO_3^- concentrations, the maximum f_{S+G_2} increased steadily, with its highest values corresponding to the highest NO_3^- concentrations. Light played a role very similar to nitrates (Fig. 5B). Below 1.5 times the euphotic zone depth (0.1% of surface light level), less than 10% of the cells were found in S + G_2. The maximum f_{S+G_2} increased steadily with decreasing depth, with the highest values being associated with surface samples. Proximity to the surface and high nitrates were not always sufficient, however, to induce high number of cells in S + G_2, as suggested by the large scatter of Fig. 5A and B, beyond the points where the threshold for either nitrate or light had been reached. For example, at Stas ZA3 and ZC1 nitrate concentrations were high in surface but f_{S+G_2} still low (filled circles, Fig. 5A). In the former case, it is likely that mixing rate was too high to allow cells to remain long enough near the surface to cycle actively. In the latter case, an unknown limiting factor was responsible for the accumulation of cells in G_1. In contrast to that observed for light and nitrates, no obvious relation emerged between f_{S+G_2} and salinity or temperature (data not shown): f_{S+G_2} was high (32%) even at relatively low temperatures (12.5°C) or low salinities (36.5‰). There was no correlation between f_{S+G_2} and cell concentration, even if only surface samples were considered (Fig. 5C). For example cell concentration was much higher at Sta. ZA1 than at Sta. ZA2, but f_{S+G_2} was much lower.

Response of prochlorophyte cell cycle distributions to nitrogen addition

To test further whether low inorganic nitrogen levels were responsible for the low fraction of prochlorophytes in S +G_2, we analysed at four stations (ZA1, ZA4, ZA6, ZC1) samples incubated in the presence of 1 μM of N-NO_3^- for 24 h. The duration of the

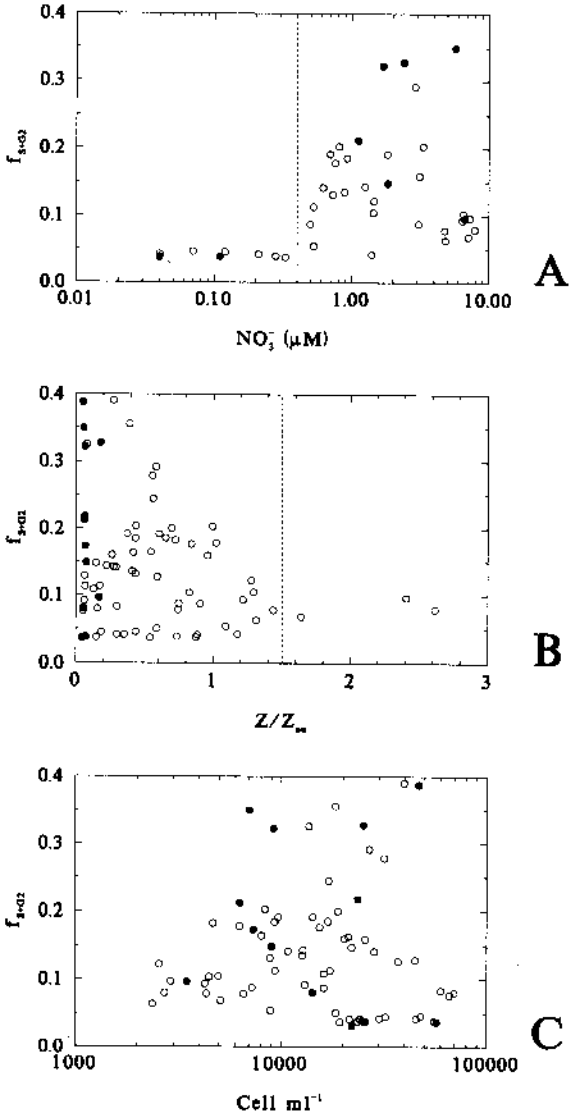

Fig. 5. Fraction of prochlorophyte cells in S + G_2 (f_{S+G_2}) as a function of nitrates (A), depth normalized to euphotic zone depth (B), and prochlorophyte cell concentration (C). Filled circles correspond to surface samples. Dashed lines in (A) and (B) indicate the limit beyond which prochlorophytes appear to cycle ($NO_3^- > 0.4\,\mu M$ and $Z < 1.5\,Z_{eu}$).

experiments alleviated ambiguities stemming from cell cycle phasing to the light–dark cycle (see below). At the oligotrophic Sta. ZA1, this treatment induced about 40% of the cells in surface to leave G_1 and enter S + G_2 (Fig. 6A). At stations where NO_3^- was initially abundant, addition of nitrogen induced very little cycling. This is illustrated by the linear relation between the increase of cells in S + G_2 after 24 h and the increase in NO_3^- resulting from the addition that was observed in surface samples at the four stations where such

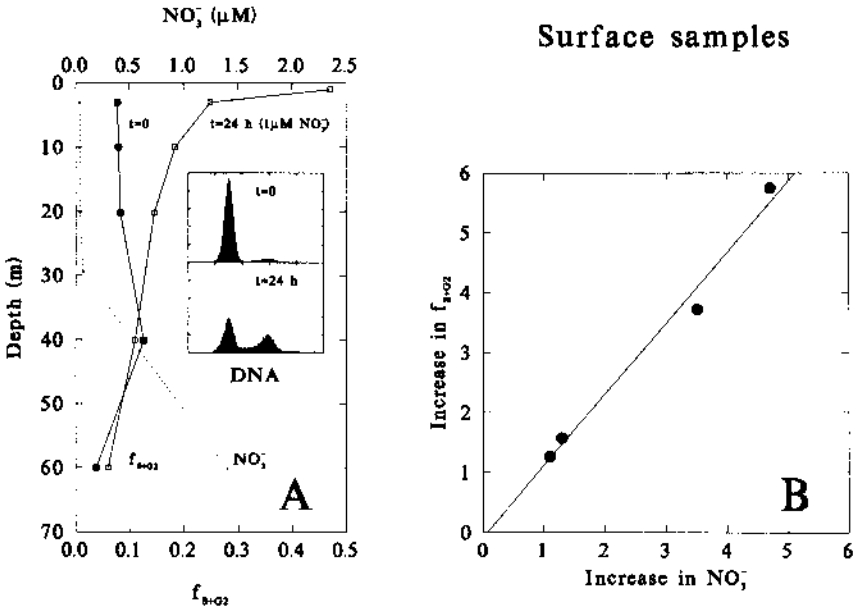

Fig. 6. Response of field prochlorophyte populations to nitrate addition (see Methods). (A) Fraction of cells in S + G_2 at the oligotrophic Sta. ZA1 before ($t = 0$, solid circles) and after ($t = 24$ h, open squares) nitrogen addition. The surface sample (3.4 m) was split between two bottles which were incubated at 1 and 3 m. The initial NO_3^- distribution is given by the dotted line. The DNA distributions in surface before and after nitrogen addition are shown in the insert. (B) Relative increase in the fraction of the cells in S + G_2 (f_{S+G_2} ($t = 24$)/f_{S+G_2} ($t = 0$)) as a function of the increase in NO_3^- resulting from 1 μM addition (($[NO_3^-]_{t=0} + 1$)/$[NO_3^-]_{t=0}$) in surface samples at four stations (ZA1, ZA4, ZA6 and ZC1). The line was drawn by hand.

experiments were performed (Fig. 6B). At Sta. ZA1, the percentage of cells induced to cycle decreased rapidly with depth. In particular below 40 m, where *in situ* NO_3^- levels were higher than in the isothermal layer, there was virtually no increase in f_{S+G_2}. At Stas ZA4 and ZC1, the effect of the incubation depth was comparable and there was virtually no observable response below 40% of the euphotic zone depth (data not shown). The addition of NH_4^+ instead of NO_3^-, or of trace concentrations (0.1 μM), yielded globally similar responses, and no differential effect of the four treatments on f_{S+G_2} could be detected (ANOVA; $P = 0.836$, $n = 72$). However, on some specific samples (e.g. at Sta. ZA1 in surface) response to 1 μM NO_3^- was stronger than to the other treatments. Surprisingly, in most samples nitrogen addition did not induce an increase in prochlorophyte cell number despite an increase in f_{S+G_2}. In particular, cell concentrations were not significantly different at $t = 0$ and $t = 24$ h with 1 μM NO_3^- added (ANOVA; $P = 0.534$, $n = 43$).

DISCUSSION

Prochlorophyte DNA histograms

Prochlorophyte populations of the northwestern Mediterranean Sea produced DNA histograms with two peaks (Fig. 2) that had coefficients of variation of the order of 9–10%,

providing sufficient resolution for cell cycle studies. Eucaryotes, which do not synthesize DNA continuously, yield such histograms where the two peaks correspond to the G_1 and G_2 + M phases of the cell cycle (VAN DILLA *et al.*, 1969). In fast-growing procaryotes, DNA synthesis is usually continuous during the whole cell cycle and the resulting DNA histograms are unimodal (STEEN and BOYE, 1980; BERNANDER and NORDSTRÖM, 1990). Flow cytometric analyses of *E. coli* (SKARSTAD *et al.*, 1983) and *Synechococcus* strain WH-8101 (ARMBRUST *et al.*, 1989) have established, however, that DNA histograms of slow-growing procaryotic populations are actually bimodal. Using an elaboration of HELMSTETTER and COOPER's (1968) model of DNA replication in *E. coli*, ARMBRUST *et al.* (1989) showed that histograms, which are such that the fluorescence ratio of the two peaks is close to 2 and the ratio of the height of the first peak to the inter-peak trough is larger than 2, are generally indicative of a DNA replication period shorter than the cell cycle and of two gaps at the beginning and end of the cell cycle. In contrast, *Synechococcus* strain PCC-6301 has been shown to yield DNA distributions with either no peak at all or multiple (i.e. more than two) peaks corresponding to 2, 3, 4, 5 . . . copies of the genome, a pattern attributed to non-synchronous initiation of replication (BINDER and CHISHOLM, 1990). Since histograms obtained for prochlorophytes in the field were never peak-less nor exhibited multiple peaks, but instead met the criteria discussed by ARMBRUST *et al.* (1989) (Fig. 2B), it is likely that prochlorophytes follow the procaryotic cell cycle model of *Synechococcus* WH-8101, with two gaps G_1 and G_2 and a discrete DNA synthesis phase S, but of course no mitotic phase M. This interpretation is supported by dual-parameter cytograms of prochlorophytes (Fig. 2A): G_2 cells have a higher chlorophyll content than G_1 cells and are therefore located probably further in their cycle, a pattern analogous to that observed for protein in eucaryotes (RONNING and LINDMO, 1983) and slow-growing *E. coli* (SKARSTAD *et al.*, 1983).

Environmental control of prochlorophyte cell cycle in situ

In eucaryotes, and more specifically photosynthetic eucaryotes, environmental factors such as light and nutrients do not have the same effect on all phases of the cell cycle. When a key factor becomes limiting, cells usually arrest in the early part of the cycle G_1 and, as a result, f_{S+G_2} decreases sharply (SPUDICH and SAGER, 1980; OLSON *et al.*, 1986). This is interpreted as the sign that light and nutrients probably are not required for transit through the late phase of the cycle and that cells, once crossing a certain point in G_1, are committed to divide (SPUDICH and SAGER, 1980). This model is not universal since, for example, some diatoms require both silica and light in late G_2 (VAULOT *et al.*, 1986, 1987; BRZEZINSKI *et al.*, 1990). The present data set suggests, however, that prochlorophytes follow the most general rules observed for photosynthetic eucaryotes.

In the wintertime northwestern Mediterranean Sea, nitrogen depletion appears to play a key role in the accumulation of cells in G_1 (Fig. 5). The implication of nitrogen is confirmed by *in situ* incubations (Fig. 6). Although negative controls, i.e. bottles incubated without added nutrients, were missing, the lack of response when significant levels of nitrogen were initially present in the samples validates these experiments. Our results are consistent with what is known in photosynthetic eucaryotes. In all species studied to date, cells depleted of nitrogen ultimately arrest at a discrete location in G_1 (OLSON *et al.*, 1986) and are released synchronously when nitrogen is resupplied (VAULOT *et al.*, 1987). The very large percentage of cells induced to cycle in S + G_2 after nitrogen

addition at Sta. ZA1 in surface waters suggests that such nutrient-induced synchrony also may exist under natural conditions. The absence of notable differences between the various treatments (NO_3^- vs NH_4^+, 1 μM vs 0.1 μM) can be explained by the fact that nitrogen when supplied above a certain threshold may act as a trigger to release arrested cells from G_1. Such a threshold effect is also suggested by the uniformly low percentage of cells in S + G_2 below 0.4 μM of NO_3^- in the field. It could be analogous to what is observed *in situ* for NO_3^- use that does not take place below 4 nM for natural populations in the equatorial Pacific (EPPLEY *et al.*, 1990). The thresholds for different cellular functions (e.g. nitrate uptake vs cell cycling), however, are probably different.

Although phosphorus, rather than nitrogen, has been implied as the key limiting factor in the Mediterranean Sea (BERLAND *et al.*, 1980), possibly because of a higher N/P inorganic ratio than in the Atlantic Ocean (MCGILL, 1965), it was unlikely that it played such a role in the situation we studied, since the inorganic N/P ratio was always lower than the Redfield ratio (data not shown).

One surprising outcome of the incubation experiments was the absence of measurable increase in cell concentration despite increase in f_{S+G_2}. This can be explained in two ways. First, if cells were actually dividing, i.e. if the increase in f_{S+G_2} translated into a parallel increase in cell division rate, the population could be maintained at a fixed level by the grazing pressure of microheterotrophs. This would mean that grazing rates would quasi-instantaneously adapt to increased division rates resulting from nutrient addition. Such rapid adaptation is rather improbable, however, as microheterotroph populations usually adapt to changes in prey density on a weekly time scale (MCMANUS and FUHRMAN, 1988). A more likely hypothesis is that cells moved synchronously from G_1 to S and G_2 in response to nitrogen addition, but none had yet divided after 24 h. Indeed, preliminary laboratory experiments indicate that total transit time through S and G_2 is always longer than 24 h in a prochlorophyte strain isolated from the Mediterranean Sea (D. Vaulot, unpublished data).

Light appears to be the other important factor modulating cell cycle distributions, as evidenced by the decrease with depth of the proportion of cells in S + G_2 both *in situ* when NO_3^- was present (Fig. 5), and in response to nitrogen addition, when NO_3^- was initially absent (Fig. 6). In culture, it has been shown that light effect on the cell cycle of photosynthetic organisms is more complex than nitrogen, since it is required either in G_1 only (in most algal groups, OLSON *et al.*, 1986) or in both G_1 and G_2 (e.g. in diatoms, OLSON *et al.*, 1986). The present field data, as well as preliminary culture experiments on a Mediterranean strain (D. Vaulot, unpublished data), suggest that prochlorophytes follow the more general model with light-dependency in G_1 only. It is interesting to note that *Synechococcus* cyanobacteria, which were closely associated with prochlorophytes in the northwestern Mediterranean Sea in winter (VAULOT *et al.*, 1990), follow different rules with light-requiring segments both in G_1 and G_2 (ARMBRUST *et al.*, 1989).

Part of the variability of f_{S+G_2} observed *in situ* could be due to differences in sampling time (e.g. dawn vs dusk) since the cell cycle of photosynthetic organisms is usually phased to the photocycle (PUISEUX DAO, 1981). Populations of the procaryote *Synechococcus* also have been observed to phase to the light–dark cycle both in culture and *in situ* (CAMPBELL and CARPENTER, 1986; WATERBURY *et al.*, 1986; ARMBRUST *et al.*, 1989; OLSON *et al.*, 1990b), and prochlorophytes appear to have a similar behavior in culture (D. Vaulot, unpublished data). However, f_{S+G_2} never varied more than two-fold at a given station sampled at different times of the day, while it varied more than 10-fold throughout the study area.

Such narrow range of f_{S+G_2} diel variation also was observed in cultures (D. Vaulot, unpublished data). Therefore, it is likely that diel changes only contribute to a small part of the f_{S+G_2} variability observed *in situ*.

Prochlorophyte population dynamics

The coherence between the *in situ* cell cycle distributions of prochlorophytes in the northwestern Mediterranean Sea and what is known about cell cycle control in photosynthetic organisms allows us to interpret f_{S+G_2} as an index of their *in situ* growth rate, and may help us to gain a better understanding of their population dynamics. The different stages through which prochlorophyte cell populations move are clearly illustrated in transect ZA (Fig. 3). When the mixed layer is very deep, nutrients are abundant but the residence time of cells near the surface is too short to allow them to grow: both cell concentration and f_{S+G_2} are low (Sta. ZA3). As water stratifies and as long as nutrients are not limiting, both residence time and growth rate increase, resulting in high values for both f_{S+G_2} and cell concentrations in the mixed layer (Sta. ZA2). Finally as nutrients become depleted, cell concentration remains high but growth rate drops sharply in surface (e.g. ZA1, ZA4). At this stage, actively growing cells can only be observed within the nitracline (Fig. 4C) where they can find sufficient nutrients. Due to light limitation, however, the proportion of cells in $S + G_2$ is much lower than when nutrients are abundant in surface. Vertical profiles of f_{S+G_2} with a maximum within the nutricline (Fig. 4C) are reminiscent of those observed in the tropical Atlantic for NO_3^- use, which, in fact, is probably attributable for a large part to prochlorophytes, as suggested by size fractionation (EPPLEY and KOEVE, 1990). The sequence of events that we described (nutrient input followed by cell division followed by nutrient depletion and cell arrest), and that is analogous to what is observed for batch cultures, explain why we did not observe any correlation between f_{S+G_2} and cell concentration (Fig. 5C). In contrast, LI and DICKIE (1991) observed a positive correlation between cell concentration and frequency of dividing cells for *Synechococcus* in the North Atlantic; they interpreted this correlation as meaning that cell populations were large (or small) as a consequence of cells growing quickly (or slowly). In our case, the highest values of f_{S+G_2} corresponded to actively growing populations with intermediate cell concentration, while the highest cell concentrations were observed at nutrient-depleted stations where cells were not actively growing. The apparent discrepancy between our observatiuons and those on *Synechococcus* (LI and DICKIE, 1991) could be explained by differences in cell cycle control by nutrients between these two procaryotes.

Winter vertical profiles of prochlorophyte cell concentrations are very similar in the Mediterranean Sea and in the Sargasso Sea, probably due to the shallow depth of the nutricline in both cases (OLSON *et al.*, 1990a). Under such conditions, the other dominant photosynthetic procaryotes, *Synechococcus*, exhibit distributions very similar to prochlorophytes (VAULOT *et al.*, 1990). In summer in the Sargasso Sea, when the nitracline deepens beyond 60 m, *Synechococcus* remain in the surface layer, while prochlorophytes occupy the bottom of the euphotic zone, the depth of their maximum cell concentration being closely correlated with the depth of the nitracline (OLSON *et al.*, 1990a). These observations led workers to hypothesize that prochlorophytes are very sensitive to nitrogen depletion, but have a better photoadaptive capacity than *Synechococcus* (OLSON *et al.*, 1990a; VAULOT *et al.*, 1990). Our data confirm that nitrogen is a key factor regulating prochlorophyte populations *in situ*, but also indicate that prochlorophytes can respond

extremely rapidly to nitrogen pulses in a manner similar to oceanic *Synechococcus* (GLIBERT *et al.*, 1986; GLOVER *et al.*, 1988). Such response capacity may explain why, as long as the nitracline is not too deep, nutrient pulses associated to local wind events (KLEIN and COSTE, 1984; EPPLEY and RENGER, 1988) or deep water formation (GASCARD, 1978) may be sufficient to trigger rapid population growth on short time scales and maintain a cell concentration maximum in surface. As the nitracline deepens, this mechanism cannot play any more and prochlorophytes can only meet their nitrogen requirement near the bottom of the euphotic zone, forming a subsurface maximum. The sharp reduction of f_{S+G_2} with depth, as well as the decrease of the response to nitrogen addition at low light levels (Fig. 6), suggests that winter Mediterranean populations cannot grow very well at low-light levels. Interestingly, in culture, a Sargasso Sea isolate had a much higher growth rate at low light than a winter isolate from the Mediterranean Sea (WEST-JOHNSRUD *et al.*, 1991), indicating that Sargasso Sea populations may grow well at the level of the deep chlorophyll maximum in summer.

Our data also indicate that, although oceanic prochlorophytes are typical of pelagic rather than neritic waters (CHISHOLM *et al.*, 1988), Mediterranean coastal populations, despite their low abundance, seemed to be actively cycling. Finally, Mediterranean populations also may have a lower temperature optimum than Atlantic ones since f_{S+G_2} was still high at 12.5°C, while very few prochlorophytes can be found north of the Gulf Stream in waters below 17°C (OLSON *et al.*, 1990a).

CONCLUSION

Cell cycle measurements such as those reported here could be easily performed on other groups of the phytoplankton with slight modifications of the staining procedure (BOUCHER *et al.*, 1991). Data on *in situ* cell cycling are extremely valuable because, in contrast to static parameters such as cell or pigment concentrations, they provide information about the dynamical status of specific field populations and their response to changes in environmental parameters. In particular, the present study demonstrates that cell cycle determinations could be a very powerful tool to study nutrient and light limitation in natural populations. Cell cycle response is much more rapid and sensitive than increase in cell number or Chl *a*, which are traditionally monitored in bioassays, the inadequacy of which is clearly illustrated by the current controversy about iron limitation of phytoplankton growth in polar regions (BANSE, 1990; MARTIN *et al.*, 1990). Quantitative interpretations require, however, extensive laboratory calibrations (e.g. OLSON *et al.*, 1986). Finally, time series of DNA distributions can be used to determine the absolute growth rate of field populations without perturbation (CARPENTER and CHANG, 1988).

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REFERENCES

ARMBRUST E. V., J. D. BOWEN, R. J. OLSON and S. W. CHISHOLM (1989) Effect of light on the cell cycle of a marine *Synechococcus* strain. *Applied and Environmental Microbiology*, **55**, 425–432.

BANSE K. (1990) Does iron really limit phytoplankton production in the offshore subarctic Pacific? *Limnology and Oceanography*, **35**, 772–775.

BERLAND B. R., D. J. BONIN and S. Y. MAESTRINI (1980) Azote ou phosphore? Considérations sur le "paradoxe nutritionnel" de la mer Méditerranée. *Oceanologica Acta*, **3**, 135–142.

BERNANDER R. and K. NORDSTRÖM (1990) Chromosome replication does not trigger cell division in *E. coli*. *Cell*, **60**, 365–374.

BINDER B. J. and S. W. CHISHOLM (1990) Relationship between DNA cycle and growth rate in *Synechococcus* sp. strain PCC 6301. *Journal of Bacteriology*, **172**, 2313–2319.

BOUCHER N., D. VAULOT and F. PARTENSKY (1991) Flow cytometric determination of phytoplankton DNA in cultures and oceanic populations. *Marine Ecology Progress Series*, **71**, 75–84.

BRZEZINSKI M. A., R. J. OLSON and S. W. CHISHOLM (1990) Silicon availability and cell cycle progression in marine diatoms. *Marine Ecology Progress Series*, **67**, 83–96.

CAMPBELL L. and E. J. CARPENTER (1986) Diel patterns of cell division in marine *Synechococcus* spp. (Cyanobacteria): use of the frequency of dividing cells technique to measure growth rate. *Marine Ecology Progress Series*, **32**, 139–148.

CARAUX D. and R. W. AUSTIN (1983) Delineation of seasonal changes of chlorophyll frontal boundaries in Mediterranean coastal waters with Nimbus-7 coastal zone color scanner data. *Remote Sensing of Environment*, **13**, 239–249.

CARPENTER E. J. and J. CHANG (1988) Species-specific phytoplankton growth rates via diel DNA synthesis cycles. I. Concept of the method. *Marine Ecology Progress Series*, **43**, 105–111.

CHISHOLM S. W., R. J. OLSON, E. R. ZETTLER, J. WATERBURY, R. GOERICKE and N. WELSCHMEYER (1988) A novel free-living prochlorophyte occurs at high cell concentrations in the oceanic euphotic zone. *Nature*, **334**, 340–343.

CHISHOLM S. W., S. L. FRANKEL, R. GOERICKE, R. J. OLSON, B. PALENIK, J. B. WATERBURY, L. WEST-JOHNSRUD and E. R. ZETTLER (1992) *Prochlorococcus marinus* nov. gen. nov. sp: an oxyphototrophic marine prokaryote containing divinyl chlorophyll *a* and *b*. *Archives of Microbiology*, **157**, 297–300.

EPPLEY R. W. and W. KOEVE (1990) Nitrate use by plankton in the eastern subtropical North Atlantic March–April 1989. *Limnology and Oceanography*, **35**, 1781–1788.

EPPLEY R. W. and E. H. RENGER (1988) Nanomolar increase in surface layer nitrate concentration following a small wind event. *Deep-Sea Research*, **35**, 1119–1125.

EPPLEY R. W., C. GARSIDE, E. H. RENGER and E. ORELLANA (1990) Variability of nitrate concentration in nitrogen-depleted subtropical surface waters. *Marine Biology*, **107**, 53–60.

FURNAS M. J. (1990) *In situ* growth rates of marine phytoplankton: approaches to measurement, community and species growth rates. *Journal of Plankton Research*, **12**, 1117–1151.

FURNAS M. J. (1991) Net *in situ* growth rates of phytoplankton in an oligotrophic, tropical shelf ecosystem. *Limnology and Oceanography*, **36**, 13–29.

GASCARD J. C. (1978) Mediterranean deep water formation, baroclinic instability and oceanic eddies. *Oceanologica Acta*, **1**, 315–330.

GIESKES W. W. C. and G. W. KRAAY (1989) Estimating the carbon-specific growth rate of major algal species groups in eastern Indonesian waters with ^{14}C labeling of taxon-specific carotenoids. *Deep-Sea Research*, **36**, 1127–1139.

GLIBERT P. M., T. M. KANA, R. J. OLSON, D. L. KIRCHMAN and R. S. ALBERTE (1986) Clonal comparisons of growth and photosynthetic responses to nitrogen availability in marine *Synechococcus* spp. *Journal of Experimental Marine Biology and Ecology*, **101**, 199–208.

GLOVER H. E., B. B. PRÉZELIN, L. CAMPBELL, M. WYMAN and C. GARSIDE (1988) A nitrate-dependent *Synechococcus* bloom in surface Sargasso Sea water. *Nature*, **331**, 161–163.

GOERICKE R. and D. J. REPETA (in press) The pigments of *Prochlorococcus marinus*: the presence of chlorophyll a_1 and b_1 in a marine procaryote. *Limnology and Oceanography*.

GOUGH L. H. (1905) Report on the plankton of the English Channel in 1903. *Report of the North Sea Fisheries Investigatory Commission (South Area)*, **1**, 325–377.

HELMSTETTER C. E. and S. COOPER (1968) DNA synthesis during the division cycle of rapidly growing *Escherichia coli* B/r. *Journal of Molecular Biology*, **31**, 507–518.

KLEIN P. and B. COSTE (1984) Effects of wind-stress variability on nutrient transport into the mixed layer. *Deep-Sea Research*, **31**, 21–37.

LANDE R., W. K. W. LI, E. P. HORNE and A. M. WOOD (1989) Phytoplankton growth rates estimated from depth profiles of cell concentration and turbulent diffusion. *Deep-Sea Research*, **36**, 1141–1159.

LANDRY M. R. and R. P. HASSETT (1982) Estimating the grazing impact of marine microzooplankton. *Marine Biology*, **67**, 283–288.

LI W. K. W. and P. M. DICKIE (1991) Relationship between the number of dividing and non-dividing cells of cyanobacteria in North Atlantic picoplankton. *Journal of Phycology*, **27**, 559–565.

MARTIN J. H., W. K. BROENKOW, S. E. FITZWATER and R. M. GORDON (1990) Yes it does: a reply to the comment by Banse. *Limnology and Oceanography*, **35**, 775–777.

MCDUFF R. E. and S. W. CHISHOLM (1982) The calculation of *in situ* growth rates of phytoplankton populations from fractions of cells undergoing mitosis: a clarification. *Limnology and Oceanography*, **27**, 783–788.

MCGILL D. A. (1965) The relative supplies of phosphate, nitrate and silicate in the Mediterranean Sea. *Rapports de la Commision Internationale pour la Mer Méditerranée*, **18**, 737–744.

MCMANUS G. B. and J. A. FUHRMAN (1988) Control of marine bacterioplankton populations: measurement and significance of grazing. *Hydrobiologia*, **159**, 51–62.

MOREL A. (1988) Optical modelling of the upper ocean in relation to its biogenous matter content (case I waters). *Journal of Geophysical Research*, **93**, 10749–10768.

NEWELL S. Y. and R. R. CHRISTIAN (1981) Frequency of dividing cells as an estimator of bacterial productivity. *Applied and Environmental Microbiology*, **42**, 23–31.

OLSON R. J., D. VAULOT and S. W. CHISHOLM (1986) Effect of environmental stresses on the cell cycle of two marine phytoplankton species. *Plant Physiology*, **80**, 918–925.

OLSON R. J., E. R. ZETTLER, M. A. ALTABET, J. A. DUSENBERRY and S. W. CHISHOLM (1990a) Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. *Deep-Sea Research*, **37**, 1033–1051.

OLSON R. J., E. R. ZETTLER, E. V. ARMBRUST and S. W. CHISHOLM (1990b) Pigment, size and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans. *Limnology and Oceanography*, **35**, 45–58.

PUISEUX DAO S. (1981) Cell-cycle events in unicellular algae. *Canadian Bulletin of Fisheries and Aquatic Sciences*, **210**, 130–149.

RIVKIN R. B. and H. H. SELIGER (1981) Liquid scintillation counting for ^{14}C uptake of single algal cells isolated from natural samples. *Limnology and Oceanography*, **26**, 780–785.

RONNING O. and T. LINDMO (1983) Progress through G_1 and S in relation to net protein accumulation in human NHIK 3025 cells. *Experimental Cell Research*, **144**, 171–179.

SKARSTAD K., H. B. STEEN and E. BOYE (1983) Cell cycle parameters of slowly growing *Escherichia Coli* B/r studied by flow cytometry. *Journal of Bacteriology*, **154**, 656–662.

SPUDICH J. and R. SAGER (1980) Regulation of *Chlamydomonas* cell cycle by light and dark. *Journal of Cell Biology*, **85**, 136–145.

STEEN H. B. and E. BOYE (1980) *Escherichia coli* growth studied by dual-parameter flow cytophotometry. *Journal of Bacteriology*, **145**, 1091–1094.

VAN DILLA M. A., T. T. TRUJILLO, P. F. MULLANEY and J. R. COULTER (1969) Cell microfluorometry: a method for rapid fluorescence measurements. *Science*, **163**, 1213–1214.

VAULOT D. and F. PARTENSKY (1989) Winter distribution of marine cyanobacteria (*Synechococcus* spp.) in the North-Western Mediterranean Sea. In: *Water pollution research reports, 13: EROS 2000 (European River Ocean System) Project Workshop*, J.-M. MARTIN and H. BARTH, editors, CEC, Brussels, pp. 136–149.

VAULOT D., R. J. OLSON and S. W. CHISHOLM (1986) Light and dark control of the cell cycle in two phytoplankton species. *Experimental Cell Research*, **167**, 38–52.

VAULOT D., R. J. OLSON, S. M. MERKEL and S. W. CHISHOLM (1987) Cell cycle response to nutrient starvation in two marine phytoplankton species. *Marine Biology*, **95**, 625–630.

VAULOT D., C. COURTIES and F. PARTENSKY (1989) A simple method to preserve oceanic phytoplankton for flow cytometric analyses. *Cytometry*, **10**, 629–635.

VAULOT D., F. PARTENSKY, J. NEVEUX, R. F. C. MANTOURA and C. LLEWELLYN (1990) Winter presence of prochlorophytes in surface waters of the northwestern Mediterranean Sea. *Limnology and Oceanography*, **35**, 1156–1164.

WATERBURY J. B., S. W. WATSON, F. W. VALOIS and D. G. FRANKS (1986) Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. *Canadian Bulletin of Fisheries and Aquatic Sciences*, **214**, 71–120.

WEST-JOHNSRUD L., B. PALENIK, S. FRANKEL, R. OLSON, E. ZETTLER, M. MCCONNEL and S. W. CHISHOLM (1991) *In vitro* growth of a marine prochlorophyte. Abstract, ASLO Meeting, Halifax, Nova Scotia, June 1991.

WILLIAMS P. J. LEB and J. 1. ROBERTSON (1989) A serious inhibition problem from a Niskin sampler during plankton productivity studies. *Limnology and Oceanography*, **34**, 1300–1305.

WOODWARD E. M. S. and N. J. P. OWENS (1989) The influence of the river Rhône upon the nutrient fluxes of the Golfe du Lion. In: *Water pollution research reports, 13: EROS 2000 (European River Ocean System) project workshop*, J.-M. MARTIN and H. BARTH, editors, CCE, Brussels, pp. 79–86.