Growth of *Prochlorococcus*, a Photosynthetic Prokaryote, in the Equatorial Pacific Ocean

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The cell cycle of *Prochlorococcus*, a prokaryote that accounts for a sizable fraction of the photosynthetic biomass in the eastern equatorial Pacific, progressed in phase with the daily light cycle. DNA replication occurred in the afternoon and cell division occurred at night. Growth rates were maximal (about one doubling per day) at 30 meters and decreased toward the surface and the bottom of the ocean. Estimated *Prochlorococcus* production varied between 174 and 498 milligrams of carbon per square meter per day and accounted for 5 to 19 percent of total gross primary production at the equator. Because *Prochlorococcus* multiplies close to its maximum possible rate, it is probably not severely nutrient-limited in this region of the oceans.

Assessment of microbial population growth rate in aquatic systems is difficult because ubiquitous grazers remove cells as quickly as they are produced. If the cell cycle of a population is in phase with the daily light cycle, cell division rates can be estimated from changes in the fractions of cells in each cell cycle stage (*1*). This approach does not require sample incubation, which is always a potential source of artifacts. We used cell cycle fractions to investigate division rates of the phytoplanktonic prokaryote *Prochlorococcus* (*2*) in the equatorial Pacific at 140°W (Table 1). Maximum cell abundances of *Prochlorococcus* occurred in general in the top 45 m and varied in a near-threefold range from 60,000 to 170,000 cells ml^{-1} (Fig. 1). Below 45 m, *Prochlorococcus* decreased in abundance but was present down to 150 m (1 April, Fig. 1). Flow cytometric DNA histograms displayed a single peak composed of cells in the G_1 phase of the cell cycle (*3*) during the late hours of night and in the early morning (Fig. 2). Around 1400 (arrowhead, Fig. 2), a distinct population of cells began to enter the S phase at 30 m and below, while cells in the surface layer (0 to 15 m) remained in G_1. By 1700, a large fraction of cells in the deeper samples had finished genomic DNA replication and entered the G_2 phase. Meanwhile, in the surface layer (0 and 15 m), cells had entered the S phase. By 2300, cells at all depths had divided and reentered the G_1phase. This pattern was very closely reproduced in each of the five diel cycles (Table 1) sampled. Analysis of the fraction of cells in the various cell cycle phases as a function of time (Fig. 3) further reveals the extremely tight cell synchrony induced by the daily light cycle. Two independent indexes of this synchrony are the very large fraction of cells found in S in the afternoon (up to 90%) and the brevity of the S and G_2 phases, which last 4 and 2 hours, respectively [as computed according to Carpenter and Chang (*1*)]. Such synchronous cell cycling, as well as the timing of cell division early in the night, differs from other reports of phasing for oceanic phytoplankton: Dinoflagellates usually divide late in the night (*4*), whereas the cyanobacterium *Synechococcus*, a close relative of *Prochlorococcus* (*5*), divides throughout the whole daylight period (*6*).

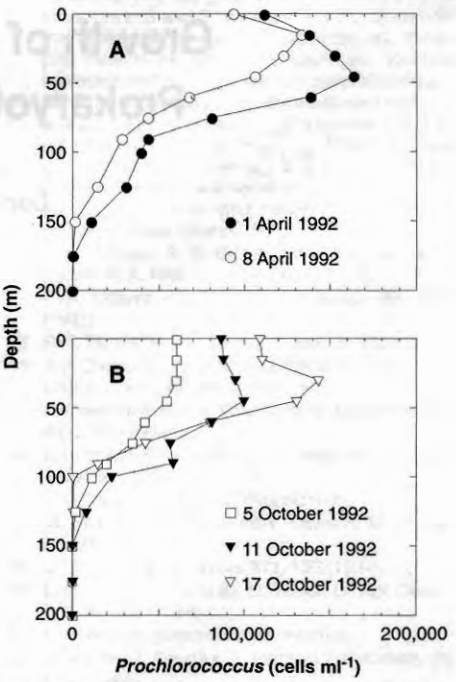

Fig. 1. Vertical profiles of *Prochlorococcus* cell concentrations at the equator (140°W) for five diel cycles sampled during the U.S. Joint Global Ocean Flux Study equatorial Pacific study in April (**A**) and October (**B**) 1992. Samples were preserved with 0.1% glutaraldehyde, frozen, and kept in liquid nitrogen (*22*). Before flow cytometric analysis, samples were stained with Hoechst 33342 (1 μg/ml) according to the method of Monger and Landry (*23*). Samples were analyzed on a Coulter EPICS 541 with the use of 400 mW of UV light. *Prochlorococcus* were discriminated from heterotrophic bacteria and other chlorophyll-containing cells as described previously (*3*).

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Two types of hypotheses have been advanced to explain cell cycle synchrony of photosynthetic organisms: direct control of light over the completion of the G_1 phase (*7*) or entrainment by a circadian clock (*8*). According to the first hypothesis, one would expect entry into the S phase to take place later at depths where less light is available. The second hypothesis would find support in the recent reports of circadian control of gene transcription and protein translation in the closely related prokaryote *Synechococcus* (*9*). If it held true in the present case, one would expect the S phase to be triggered uniformly at least over part of the water column. However, time of entry into the S phase was not completely uniform over the whole water column, occurring earlier at depth with a lag of about 3 hours between top and bottom (Fig. 2). Therefore, both hypotheses are unsatisfactory and further experimental work is needed to interpret the pattern observed in the ocean. The delay of S phase in the surface layer relative to that at 30 m may be a protective mechanism to prevent exposure of replicating DNA to high midday irradiances [especially ultraviolet (UV)]. Indeed, this delay results in cells replicating their DNA at the surface and at 30 m at equivalent light intensities on the order of 300 microeinsteins (μE) m^{-2} s^{-1} (1 E is equal to 1 mol of photons).

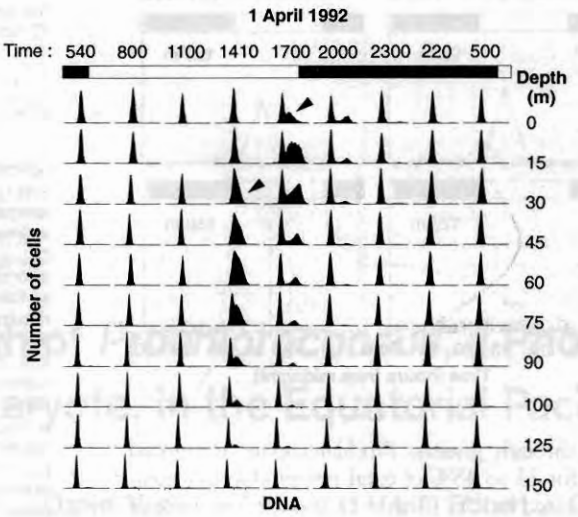

Fig. 2. Change in *Prochlorococcus* cell cycle distributions for a depth profile at the equator (140°W) during one diel cycle (1 April 1992). In each small panel, the abscissa corresponds to cell DNA content and the ordinate to cell number. The major peak corresponds to G_1 cells, whereas the minor peak on the right (for example, t = 2000) corresponds to G_2 cells. Arrowheads point to the initiation of the S phase, at 1410 (below 30 m) and at 1700 (above 30 m).

Table 1. Rate estimates integrated over the water column at the equator in the Pacific (140°W).

Date	Conditions*	*Prochlorococcus* division rate μ_{cc} integrated† (day^{-1})	*Prochlorococcus* production integrated‡ (mg of C m^{-2} day^{-1})	Total production integrated§ (mg of C m^{-2} day^{-1})		*Prochlorococcus* (% of production integrated)	
				Net	Gross	Net	Gross
1 April 1992	Warm El Niño	0.51	498	1033	2583	48	19
8 April 1992	Warm El Niño	0.63	347	981	2453	35	14
5 October 1992	Cold tongue	0.58	174	1385	3463	13	5
11 October 1992	Cold tongue	0.60	322	1843	4608	17	7
17 October 1992	Cold tongue	0.53	265	1571	3928	17	7

*According to Murray *et al.* (*11*). †Estimates of integrated division rates were computed as $\mu_{cc} = \ln\left[\int_0^{200} N_{1700}(z) \cdot \exp[\mu_{cc}(z)] \cdot dz / \int_0^{200} N_{1700}(z) \cdot dz\right]$, where z is the water depth, $\mu_{cc}(z)$ is obtained from Fig. 4, and N_{1700} is the cell concentration just before division. Such a procedure, which weighs division rates by cell concentrations just before division, is more accurate than a simple depth-averaged rate. ‡Depth-integrated daily production rates for *Prochlorococcus* were computed from the estimated production at each depth, as $P = \int_0^{200} C_{cell} \cdot N_{1700}(z) \cdot \left\{\exp[\mu_{cc}(z)] - 1\right\} \cdot dz$, where C_{cell} is the intracellular carbon content of *Prochlorococcus* estimated as 53 fg of C per cell (*20*). §Depth-integrated daily net production rates for the total community were computed from ^{14}C estimates (*21*). Gross production was obtained from net production divided by 0.4 (*18*).

We estimated the specific growth rate (μ_{cc}) from the analysis of cell cycle timing (*1*) with less than 19% error on average (*10*). Maximum estimated growth rates in excess of one doubling per day (0.73 to 0.93 day^{-1}) occurred at 30 m (Fig. 4) where cells received 18% of the surface irradiance (Fig. 4), which is equivalent to a constant irradiance of 220 μE m^{-2} s^{-1} for 12 hours. Growth rates were less near the surface (0 to 15 m) and decreased continuously with increasing depth below 30 m. Cells at 150 m, where only 0.02% of the surface irradiance was available, divided at about 0.1 day^{-1} (generation time ≈ 7 days). Vertical profiles of μ_{cc} were very similar for the five diel cycles sampled (Fig. 4). A notable increase in μ_{cc} was observed in the surface layer, but not below 30 m, during the passage of a tropical instability wave on October 11 (*11*). Rates integrated over the whole water column (Table 1) averaged 0.58 ± 0.05 day^{-1}.

Growth rates for the equatorial phytoplankton community as a whole, indirectly deduced from ^{14}C productivity measurements and phytoplankton carbon estimates [0.6 to 0.7 day^{-1} at the surface (*12*) and 0.47 day^{-1} depth-integrated (*13*)] are similar to the *Prochlorococcus* growth rates we measured (Table 1). Moreover, the photosynthetic assimilation ratio in the equatorial Pacific (*12*, *14*) is reduced near the surface, as is the *Prochlorococcus* growth rate. In contrast, maximum growth rates for *Prochlorococcus* in the Sargasso Sea, estimated from ^{14}C labeling of divinyl–chlorophyll a, are lower on average [0.1 to 0.5 day^{-1} (*15*)] than ours.

The maximum growth rates that we estimated are slightly higher than those reached in culture by *Prochlorococcus* strains isolated from the Mediterranean and Sargasso Seas (*16*). Rates in excess of one doubling per day (0.85 day^{-1}) have been observed (*17*) for isolates from the equatorial Pacific exposed to 14 hours of light (natural populations at the equator are only exposed to 12 hours of light per day). We suggest that *Prochlorococcus* cells are dividing at or near their maximum rate at 30 m in the equatorial Pacific, and therefore that their growth is probably not severely limited by nutrient deficiency. The division rate below 30 m is limited by light and is photoinhibited near the surface.

The use of an estimate for the intracellular carbon content of *Prochlorococcus* allowed us to calculate *Prochlorococcus*'s daily integrated production rate, which varied between 174 and 488 mg of C m^{-2} day^{-1} (Table 1). This estimate is probably closer to gross than net production because, although it does not include assimilated carbon that is excreted as dissolved organic carbon, it incorporates carbon that is later recycled

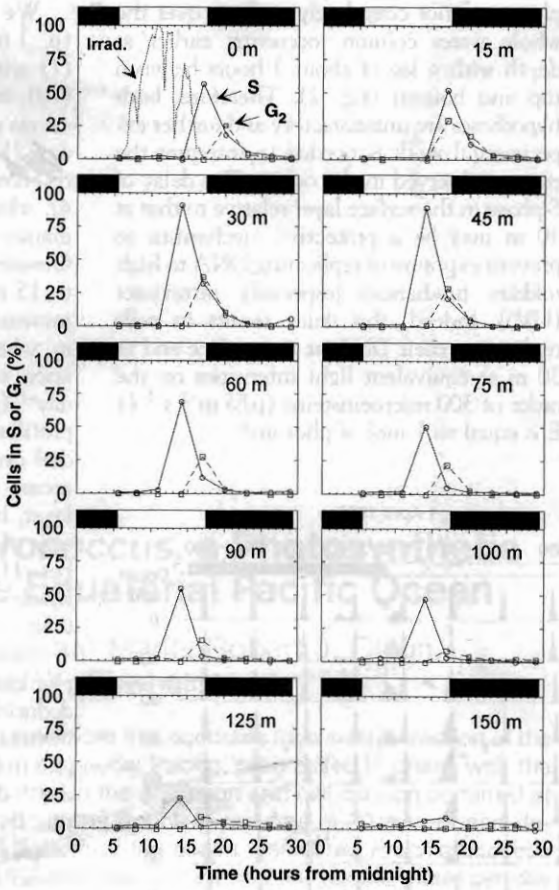

Fig. 3. Percentage of *Prochlorococcus* cells in S (circles, solid line) and G_2 (squares, dashed line) for a depth profile at the equator (140°W) during one diel cycle (1 April 1992). Cell fractions were computed from DNA distributions with the use of MCYCLE software (Phoenix Flow Systems, San Diego, California). Superimposed dashed line at 0 m corresponds to surface solar radiation (from 0.3 to 3 μm) expressed in W m^{-2} × 10 (*24*).

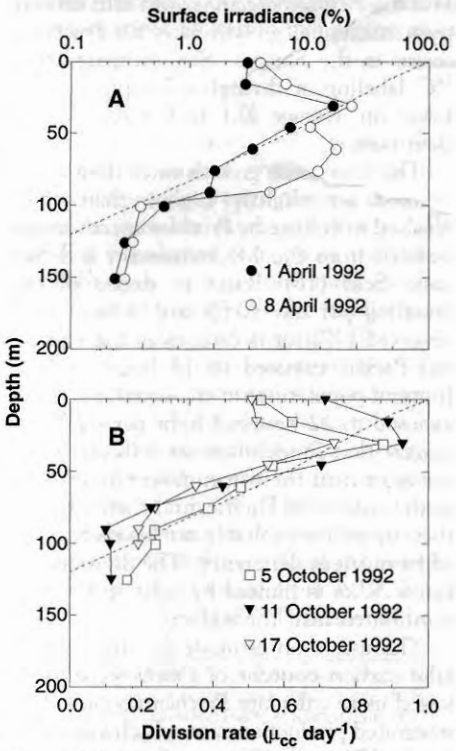

Fig. 4. *Prochlorococcus* division rate (μ_{cc} day^{-1}) estimated from cell cycle phase variation (*1, 10*) as a function of depth for five diel cycles at the equator (140°W) in April (**A**) and October (**B**) 1992. Dashed line corresponds to percent of photosynthetic radiation available at the surface (*21*).

through grazers. *Prochlorococcus* accounted for 13 to 48% of total net production measured by ^{14}C (Table 1) and for 5 to 19% of total gross production (*18*). This contribution was much higher under warm El Niño (more oligotrophic) conditions, because total production was lower and *Prochlorococcus* biomass (and therefore production) was higher.

This study reveals two dimensions of the ecology of *Prochlorococcus*. First, cell division is strongly entrained to the diel light-dark cycle, even at depths where light intensity does not sustain high division rates. Second, cells are multiplying rapidly, close to their maximum potential growth rates. This suggests that nutrients (such as iron) are not severely limiting *Prochlorococcus* growth in the equatorial region. This suggestion does not contradict the observed phytoplankton response to a recent iron fertilization experiment (*19*), because that response was due primarily to the non-*Prochlorococcus* fraction of the community, that is, *Synechococcus* cyanobacteria and eukaryotic algae.

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10. Briefly, the combination of the S and G_2 phases was chosen as the terminal phase and its duration (t_{S+G2}) was computed as twice the distance between the peak of cells in S and the peak of cells in G_2 (see Fig. 3). Because the sampling frequency for a given diel cycle was low (every 3 hours), we obtained a more robust estimate of t_{S+G_2}, by assuming it did not vary for a given cruise: At each depth, all diel samples from a given cruise were pooled together. For both cruises, t_{S+G_2} was equal to 6 hours from the surface down to 75 m and then increased up to 12 hours at 125 m. The μ_{cc} was computed as $\mu_{cc} = \frac{1}{n \cdot t_{S+G_2}} \sum_{i=1}^{n} \ln[1 + f_{S+G_2}(i)]$, where n is the number of samples taken during a 24-hour period and $f_{S+G_2}(i)$ is the fraction of cells in S + G_2 for sample i. Error in μ_{cc} stems mainly from error in estimating t_{S+G_2}. For example, an error of 2 hours in t_{S+G_2} would translate into a 30% error in μ_{cc}. This error can be more precisely assessed in a few cases as follows. The very good synchrony and the single maximum in S and G_2 we observed (Fig. 3) indicate that at most one division took place per day. Therefore, rates in excess of 0.69 day^{-1} are obviously overestimated ($\mu_{cc} \geq \mu$). We can compute independently an absolute minimum for the division rate (*27*): $\mu \geq \mu_{min} = \ln[1 + \max(f_{S+G_2})]$, a formula that does not rely on an estimate of t_{S+G_2}. Therefore, when $\mu_{cc} \geq 0.69$ day^{-1}, then $\mu_{cc} \geq \mu \geq \mu_{min}$, and the maximum error on μ is equal to ($\mu_{cc} - \mu_{min}$). In our case, this maximum error ranged from 7 to 33%, with an average value of 19% (n = 11).
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