

Prochlorococcus growth rate and contribution to primary production in the equatorial and subtropical North Pacific Ocean

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ABSTRACT: DNA synthesis and cell division of *Prochlorococcus* are tightly synchronized to the daily light cycle, therefore, cell division rates can be estimated from the fraction of cells in each cell cycle stage during a 24 h sampling period. The total mortality rate of *Prochlorococcus* can also be estimated from the difference between the observed cell abundance and the expected cell number projected from growth rate in that sampling period, providing an estimate of grazing impact. Growth and mortality rates of *Prochlorococcus* were investigated at 2 stations in the equatorial Pacific, as well as Station ALOHA in the subtropical North Pacific Ocean. Growth rate of *Prochlorococcus* remained high at all sites independent of the nitrate concentration. The maximum growth rate (up to 1 doubling d⁻¹) occurred at 70 m depth at the western equatorial Pacific site (166° E) and at 40 to 45 m at the eastern equatorial Pacific site (150° W) and at Station ALOHA (22° 45' N, 158° W). Total mortality rates were roughly balanced by *Prochlorococcus* growth at all stations. Because of the phased cell cycle and continuous (if not evenly distributed) mortality, the abundance of *Prochlorococcus* at each depth could vary up to 2-fold between afternoon and midnight. *Prochlorococcus* production was estimated to contribute 9 and 39% to the total gross primary production in the eastern and western equatorial Pacific, respectively, and up to 82% in the subtropical North Pacific Ocean at Station ALOHA. Our results suggest *Prochlorococcus* are not severely nutrient-limited in the oligotrophic environment. Rapid nutrient recycling by grazing activity permits *Prochlorococcus* to contribute a significant fraction of the total primary production.

KEY WORDS: *Prochlorococcus* · Cell cycle · Growth · Grazing · Production · Pacific Ocean · Flow cytometry

INTRODUCTION

Picophytoplankton, including *Prochlorococcus*, *Synechococcus* and picoeucaryotes (<2 µm), is the dominant contributor to biomass and primary production in the subtropical and tropical ocean (Li et al. 1983, Platt et al. 1983, Peña et al. 1990, Le Bouteiller et al. 1992, Campbell et al. 1994). Recent studies indicate that picoeucaryotes dominate the biomass and productivity of picophytoplankton in most regions of the world ocean (see Li 1995), except the central North Pacific

where *Prochlorococcus* accounted for 64% of biomass (Campbell et al. 1994) and up to 44% of gross primary production (Vaulot 1995). The grazing of heterotrophic flagellates and ciliates on *Prochlorococcus* and other picoplankters is substantial. Mortality due to grazing is essentially equivalent to the growth of picophytoplankton and probably controls the temporal variation of the phytoplankton population (Banse 1992, Cullen et al. 1992, Landry et al. 1995, Liu H. et al. 1995). Existing methods for estimating population-specific growth and grazing rates include the dilution (Landry & Hassett 1982) and selective metabolic inhibitor (Fuhrman & McManus 1984) techniques. These methods have the same disadvantage, i.e. they each use long-term (usually 24 h) incubation which can be a major source of uncertainty. Apart from the bottle effect, metal con-

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tamination can be introduced during sampling and handling procedures (Fitzwater et al. 1982). Serious error can also be caused by the inability to mimic the intensity and spectral characteristics of submarine light properly (Laws et al. 1990, McManus 1995).

Two prokaryotic cell cycle models, the fast growth and the slow growth, have been proposed (Cooper & Helmstetter 1968). In fast-growing prokaryotes, DNA synthesis is usually continuous during the whole cell cycle and the resulting DNA histograms are unimodal or multimodal. The slow growth case applies when the generation time of the cells is less than the sum of the time required for DNA replication and the time between the termination of replication and cell division. Consequently, the resulting DNA frequency distributions are bimodal. The first peak corresponds to cells in G_1 (prior to the start of chromosome replication), the second peak corresponds to cells in G_2 (those having finished DNA replication but not yet divided), and the intermediate part of the distribution corresponds to the cells in S (those in the midst of DNA replication). The observation of bimodal DNA histograms and synchronous cell-division in *Prochlorococcus* (Vaulot & Partensky 1992, Vaulot et al. 1995) permits growth rate estimates of *in situ* *Prochlorococcus* populations to be made based on the fraction of cells in each cell cycle stage during a 24 h sampling period (Carpenter & Chang 1988). Estimation of growth rate with this approach thus avoids errors introduced by incubation. Because this estimate of growth rate is independent of grazing impact and other losses, the total mortality rate of *Prochlorococcus* can also be calculated from the difference between the observed cell abundance and the cell number projected from growth rate in that sampling period. We used the diel cell cycle approach at time-series stations in the equatorial Pacific (166° E and 150° W) and at ~100 km north of Oahu, Hawaii, USA, at Station ALOHA (22° 45' N, 158° W) to investigate the growth and mortality rates of *Prochlorococcus* and its contribution to primary production.

MATERIALS AND METHODS

Samples were collected from 2 equatorial time-series stations (166° E and 150° W, hereafter referred to as W-EqPac and E-EqPac, respectively) during the French JGOFS FLUPAC cruise in October 1994 and during 2 cruises in the subtropical North Pacific at Station ALOHA (22° 45' N, 158° W) in September 1993 and July 1994 (HOT49 and HOT55, hereafter referred to as ALOHA Sept 93 and ALOHA July 94, respectively). Using Niskin bottles mounted on a CTD rosette, 9 depths throughout the 150 m euphotic zone

were sampled at frequent intervals over a 24 h period. Although the time interval between sampling varied from 1.5 to 5 h, the most frequent sampling took place from noon through midnight because *Prochlorococcus* DNA synthesis and cell division occurs during this period (Vaulot et al. 1995). Samples (1 ml) were preserved with 0.2% (final concentration) paraformaldehyde in cryovials, quickly frozen in liquid nitrogen and stored at -80°C until flow cytometric analysis.

Samples were analyzed upon return to the laboratory using a Coulter EPICS 753 flow cytometer equipped with two 5 W argon lasers and an MSDS automatic sampler. The flow cytometer was set up for UV (225 mW) and 488 nm (1 W) colinear analysis. Hoechst 33342 (DNA-specific fluorochrome) was added to thawed sample at a final concentration of 1 µg ml⁻¹ and incubated for 1 h at room temperature in the dark. To achieve a better coefficient of variation (CV) for fluorescence, a slower delivery rate (10 µl⁻¹) was used. CV's for both G_1 and G_2 peaks ranged from 6 to 8% for the *Prochlorococcus* from the upper 100 m of the water column and were not greater than 10% for those from the bottom of the euphotic zone at all locations. For statistical purposes, 100 to 300 µl of samples was analyzed to collect more than 10 000 events of a gated *Prochlorococcus* population, except for the samples below 125 m which contained fewer *Prochlorococcus*, making 10 000 counts impractical. Six parameters (log integrated chlorophyll and DNA fluorescence, forward and 90° light scatter signals, linear integrated and peak DNA fluorescence) were collected in list mode for *Prochlorococcus* and analysed using the CYTOPC software (Vaulot 1989).

The *Prochlorococcus* cell cycle is analogous to the eucaryotic cell cycle. It exhibits a bimodal DNA distribution, i.e. DNA histograms display 2 peaks corresponding to the initial growth phase, G_1 , and the late growth phase, G_2 , separated by a discrete DNA synthesis phase S (Vaulot & Partensky 1992). One-parameter histogram files containing the DNA fluorescence data for *Prochlorococcus* were analyzed using MCYCLE software (Phoenix Flow Systems, San Diego, CA, USA) and cell fractions in G_1 , S and G_2 phases were computed from DNA distributions. The combination of the S and G_2 phases was chosen as the terminal phase and its duration (t_{S+G_2}) was computed as twice the distance between the peak of cells in S and the peak of cells in G_2 (Carpenter & Chang 1988). Because the distance between the 2 peaks is obviously affected by the time interval between sampling, the inaccuracy in the estimation of t_{S+G_2} is the major source of error for the growth rate calculation. Since hourly CTD casts were not possible, our solution was to collect surface bucket samples more frequently, i.e. every hour during *Prochlorococcus* DNA replication and cell division

(15:00 to 24:00 h local time). Results from 2 successive days verified a 3 h distance from the peak of S to the peak of G_2 (Fig 1). An average t_{S+G_2} of 6 h was used for *Prochlorococcus* in the upper 100 m in our calculation, which is the same time period used by Vaultot et al. (1995). Because t_{S+G_2} tended to be longer for the *Prochlorococcus* living under very low light intensity, observed time interval between the peaks of cells in S and G_2 phases was used for samples taken below 100 m.

Growth rate (μ , d^{-1}) was computed as

$$\mu = \frac{1}{t_{S+G_2}} \int_0^{24} \ln[1 + f_{S+G_2}(t)] \quad (1)$$

where $f_{S+G_2}(t)$ is the fraction of cells in S+ G_2 at time point t in the 24 h sampling period. The formula described in Vaultot et al. (1995), a modification of McDuff & Chisholm (1982), is only valid when all cells have the same duration of a terminal event, t_d , and requires fixed sampling intervals (Vaultot 1992). It will dramatically overestimate the division rate if samples are taken more frequently during the high S and G_2 period. The formula we used here computes the area under the percentage curve of S+ G_2 and, therefore, is not affected by the change in sampling frequency.

Integrated division rates (μ_{int}) in the upper 150 m of the water column were computed as

$$\mu_{int} = \ln \left[\int N_{initial}(z) e^{\mu(z)} dz / \int N_{initial}(z) dz \right] \quad (2)$$

where z is the water column depth; $\mu(z)$ is the growth rate calculated from Eq. (1) and $N_{initial}$ is the cell concentration just before division (from 16:00 h for the deep layer to as late as 20:00 h for surface samples). This approach weights division rates by cell concentrations just before division and is more accurate than a simple depth-averaged rate.

We calculated *Prochlorococcus* mortality rates (m , d^{-1}) from

$$m = -\ln(N_{24}/N_0) + \mu \quad (3)$$

where N_0 and N_{24} are the concentration of *Prochlorococcus* at the beginning and end of the 24 h sampling period, respectively. The mortality rate estimated from Eq. (3) is based on the assumption that other loss terms, such as sinking and advection, are negligible and the effect of patchy distribution does not exist at the sampling site. Assuming we were sampling the same waters during the entire 24 h sampling period, the integrated mortality rates (m_{int}) in upper 150 m of the water column were calculated from

$$m_{int} = -\ln \left[\int N_{24}(z) dz / \int N_0(z) dz \right] + \mu_{int} \quad (4)$$

Depth-integrated carbon production ($mg\ C\ m^{-2}\ d^{-1}$) of *Prochlorococcus* was computed using the carbon conversion factors from Campbell et al. (1994). Total

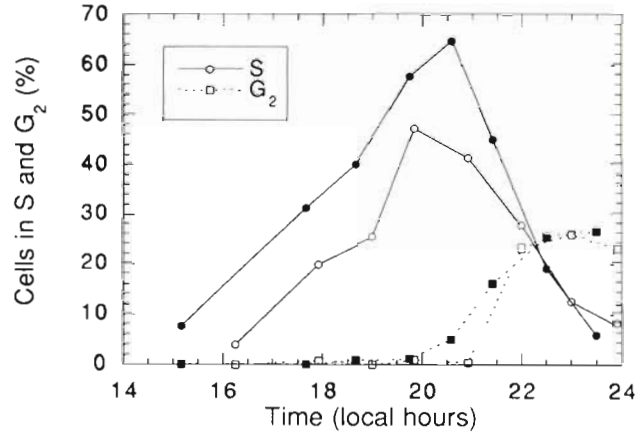


Fig. 1. Percentage of *Prochlorococcus* cells in S (circles) and G_2 (squares) phases for the samples taken from the surface waters of Station ALOHA ($22^{\circ} 45' N$, $158^{\circ} W$) on July 29 (○, □) and July 30 (●, ■), 1995

gross primary production was converted from ^{14}C data by multiplying by 1.55 (Bender et al. 1992) to account for the fraction of *Prochlorococcus* contribution.

RESULTS AND DISCUSSION

The western equatorial Pacific (W-EqPac; $166^{\circ} E$) is an oligotrophic environment with a high temperature ($\sim 30^{\circ}C$), low salinity (~ 33.9 psu) surface mixed layer extending to ~ 90 m. Nitrate and nitrite were not detectable above the thermocline and a strong, deep (90 m) chlorophyll maximum coincided with the nitracline (Le Borgne et al. 1995). The eastern equatorial Pacific (E-EqPac; $150^{\circ} W$) is a mesotrophic habitat featuring measurable nutrients and higher primary production in the surface mixed layer. However, during our investigation, the existence of a deep surface mixed layer (~ 100 m) suggested developing El Niño conditions (Barber & Chavez 1983, Liu W. T. et al. 1995). The abundance of *Prochlorococcus* at W-EqPac was about twice as high as that in E-EqPac (Fig. 2). However, the abundance of other picophytoplankton, i.e. *Synechococcus* and picoeucaryotes, was significantly higher at E-EqPac (Le Borgne et al. 1995). As representative of the oligotrophic North Pacific subtropical gyre, Station ALOHA is characterized by a nutrient-depleted surface mixed layer and a low standing stock of organisms and primary production (Karl et al. 1995). *Prochlorococcus* dominates the picophytoplankton biomass with an average depth-integrated abundance of about 2×10^9 cells cm^{-2} (Campbell & Vaultot 1993) that varies 2-fold annually with a maximum in summer which usually coincides with a deeper nitracline and shallower mixed layer (Campbell et al.

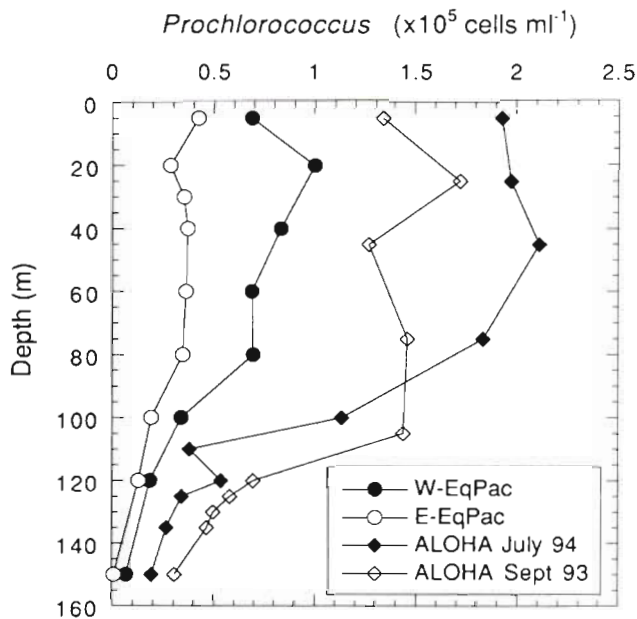


Fig. 2. Vertical profile of *Prochlorococcus* cell concentrations at W-EqPac (0°, 166° E) and E-EqPac (0°, 150° W) stations in October 1994 and in the subtropical North Pacific Ocean at Station ALOHA (22° 45' N, 158° W) in September 1993 (HOT49) and July 1994 (HOT55). Data shown here are *Prochlorococcus* cell concentrations just before cell division (~16:00 h, local time)

1996). Our investigation was conducted during summer when the mixed-layer depth was 24 to 41 m and the nitracline was ~125 m (Table 1). The vertical profiles of *Prochlorococcus* cell concentrations at each station show a subsurface peak at all stations except E-EqPac (Fig. 2).

We observed a diel DNA distribution pattern similar to the previous report by Vaultot et al. (1995) at both locations. In the relatively nutrient-rich water at E-EqPac, cells began to enter the S phase between 11:30 and 13:30 h and the G₂ phases between 16:00 and 17:30 h, at 20 m and below. Phasing was delayed by about 2.5 h in the surface waters. At the oligotrophic W-EqPac Station, this delay extended to 40 m, possibly because of the deeper euphotic zone. At Station ALOHA, the starting time for both S and G₂ phases in the upper and lower euphotic layer were further delayed by 1 to 2 h. No cells in the surface waters entered the S phase until sunset. The delay in the surface water may be a protective mechanism for avoiding high irradiance as proposed by Vaultot et al. (1995).

The estimated *Prochlorococcus* growth rates for all stations are shown in Fig. 3. Maximum growth rate ranged from 0.64 to 0.78 d⁻¹, but occurred at different depths. Maximum growth rate occurred at 70 m at the oligotrophic W-EqPac Station, 40 m at the mesotrophic E-EqPac, and 45 m at Station ALOHA during both cruises. The difference between these stations may be

Table 1 A comparison of *Prochlorococcus* cell concentration, growth and mortality rates, carbon production and contribution to the total primary production. All depth-integrated data are for the upper 150 m of the water column

Region	Equatorial Pacific		Subtropical North Pacific	
	Western	Eastern	ALOHA	
Station	0°, 167° E	0°, 150° W	22° 45' N, 158° W	
Location	0°, 167° E	0°, 150° W	22° 45' N, 158° W	
Cruise	FLUPAC	FLUPAC	HOT49	HOT55
Date	Oct 1994	Oct 1994	Sept 1993	Jul 1994
Environment	Oligotrophic	Mesotrophic	Oligotrophic	Oligotrophic
Surface NO ₃ concentration (μM) ^a	0	2.8	0	0
Nitracline depth (m) ^a	90		126	125
Integrated NO ₃ conc. (μmol cm ⁻²)	57	92	5.3	2.8
Integrated chl <i>a</i> conc. (mg m ⁻²) ^a	25.2	28.6	19.1	22.8
Integrated <i>Prochlorococcus</i> abundance (cm ⁻²)				
Minimum	8.35 × 10 ⁸	3.97 × 10 ⁸	1.63 × 10 ⁹	1.95 × 10 ⁹
Maximum	1.15 × 10 ⁹	6.74 × 10 ⁹	2.16 × 10 ⁹	2.61 × 10 ⁹
Integrated growth rates (d ⁻¹)	0.61	0.60	0.53	0.57
Depth of maximum μ (m)	70	40	45	45
Integrated mortality rates (d ⁻¹)	0.59	0.47	0.45	0.54
Integrated <i>Prochlorococcus</i> production (mg C m ⁻² d ⁻¹)	409	183	620	743
Integrated total primary production (mg C m ⁻² d ⁻¹)				
¹⁴ C ^a	670	1330	541	584
Gross	1039	2062	839	905
<i>Prochlorococcus</i> contribution to gross carbon production (%)	39	9	74	82

^aNutrient, chlorophyll and primary production data for Station ALOHA were obtained from the HOT data base via ftp (mana.soest.hawaii.edu). Data for FLUPAC cruise were obtained from the data center in Orstom, Centre de Nouméa with the assistance of P. Polidori except for the primary production data that were provided by A. Le Bouteiller

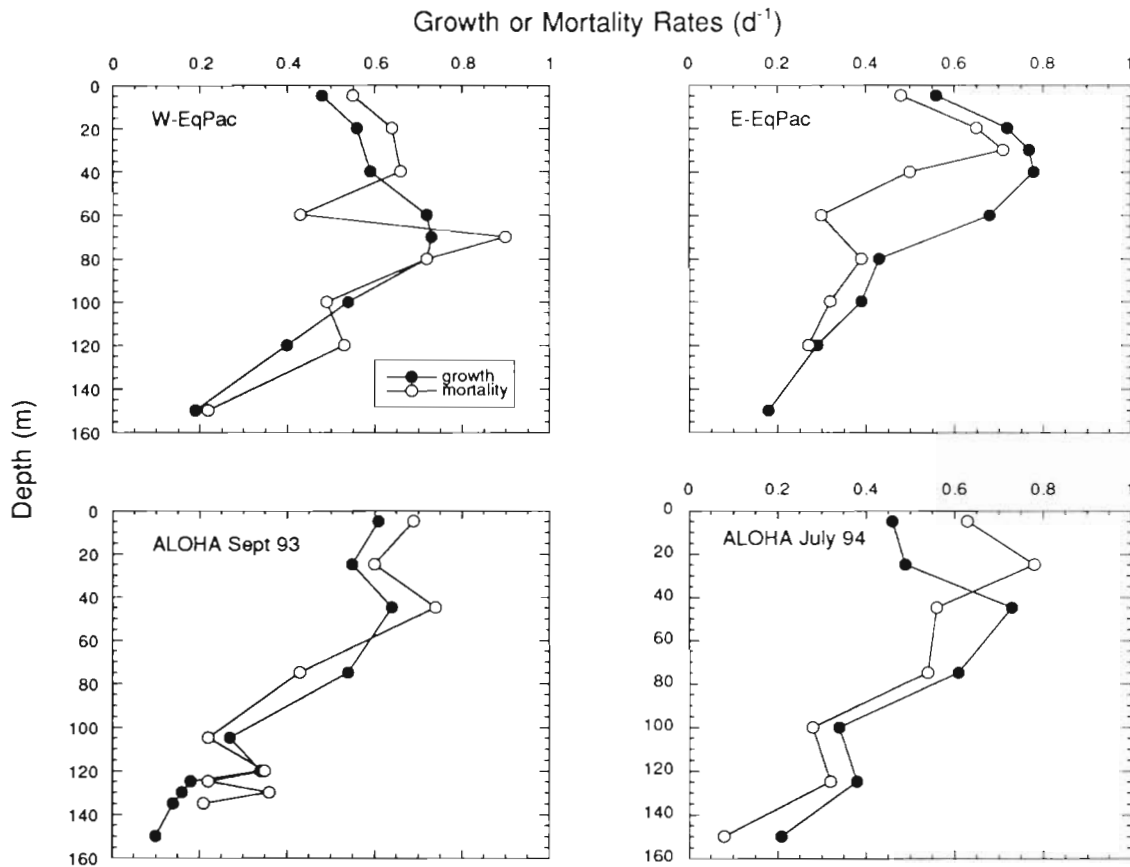


Fig. 3. *Prochlorococcus* growth and mortality rates estimated from the diel cell cycle phase variation as a function of depth for each studied site

explained by the differences in the ecophysiological characteristics of the dominant *Prochlorococcus* population in the surface layer. If we plot the *Prochlorococcus* growth rates from the 4 experiments as a function of the percentage of photosynthetically active radiation (PAR) transmitted to the corresponding depth, we find that the maximum *Prochlorococcus* growth rate occurred at ~15% of the surface irradiance, except at W-EqPac where the maximum *Prochlorococcus* growth rate was at the 5% light level. This physiological difference may be evidence of different *Prochlorococcus* species. The integrated growth rates for the upper 150 m of the water column were quite similar at all locations (Table 1) and corresponded to previous estimates at 140° W (Vaulot et al. 1995, Binder et al. 1996). Together, high growth rate and abundance in these oligotrophic waters (Table 1) imply that *Prochlorococcus* was not nutrient-limited at the oligotrophic W-EqPac Station or at Station ALOHA.

The mortality rates calculated from Eqs. (3) & (4) give the total loss of *Prochlorococcus* biomass. The estimated total mortality rates of *Prochlorococcus* essentially matched growth rates at most depths at each

station (Fig 3). However, this 'match' is only a reflection of relatively constant cell numbers at the same time of the day because our mortality estimation is related to the growth rate estimates. Although protozoan grazing is the most important fate of *Prochlorococcus* (Landry et al. 1995, Liu H. et al. 1995), other losses such as lysis due to virus infection could be significant, especially in the high temperature, high solar irradiance tropical waters (Cottrell & Suttle 1995). It is estimated that an average of 3% of *Synechococcus* populations, a close relative of *Prochlorococcus*, are lysed daily by viruses (Suttle 1994). Fuhrman & Noble (1995) found approximately equal significance of viral lysis and protist grazing for bacterial mortality in coastal waters.

Because of the phased growth and balanced grazing, the abundance of *Prochlorococcus* in the water column also varies over a 24 h period. We obtained the diel pattern of cell concentration and all fluorescence and light scatter parameters for *Prochlorococcus* from each station. Although there was noise at some individual depths that was probably due to the movement of the research vessel and horizontal variation or patchiness

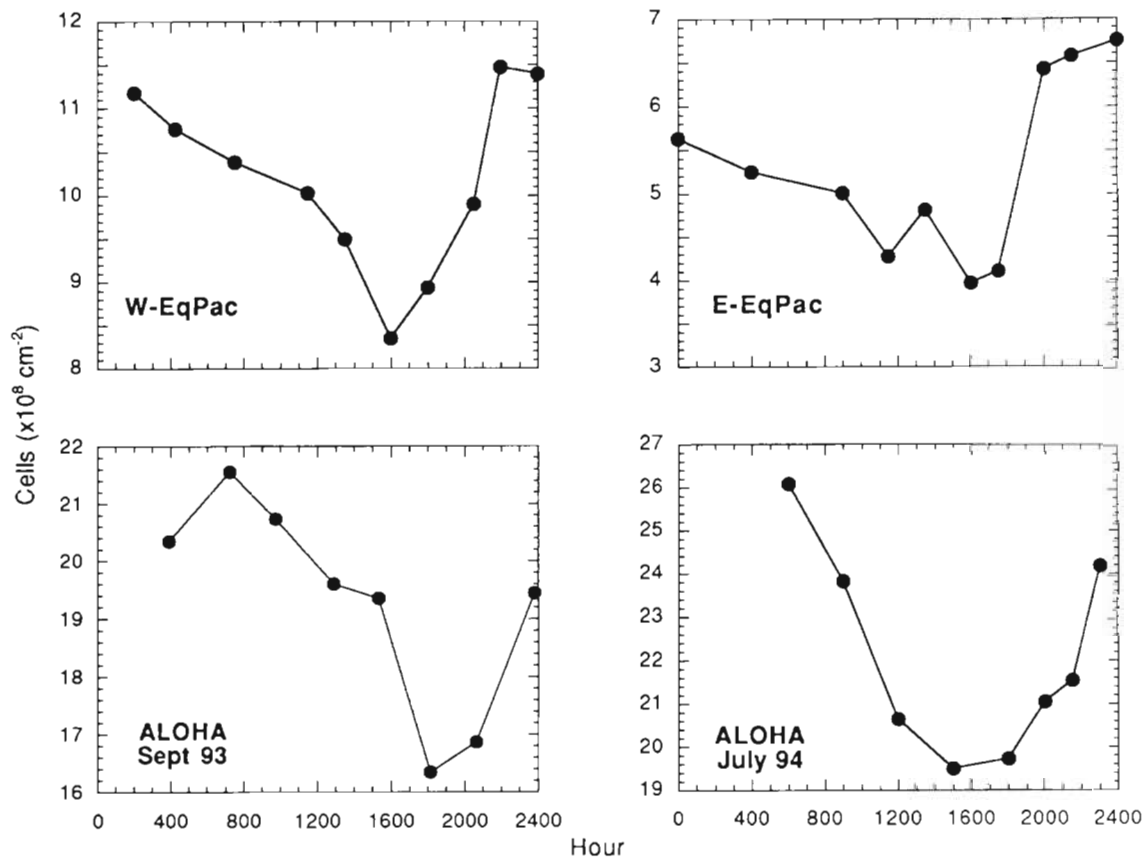


Fig. 4. Daily variation of depth-integrated *Prochlorococcus* cell concentrations for all sampling sites

in the vicinity of the study area, the variation of the depth-integrated *Prochlorococcus* cell concentration through the 24 h sampling period showed the same pattern at all stations (Fig. 4). The maximum cell concentration occurred around midnight. Cell concentration then decreased and began to increase again between 16:00 and 18:00 h. Although the integration over the water column mixed up the 2 to 3 h difference in cell cycle progress, it clearly indicated synchronized growth of *Prochlorococcus*. If the mortality of *Prochlorococcus* caused by microzooplankton grazing and other losses is constant throughout the day, the ratio of the minimum to maximum cell concentration in a day can be predicted by a simple model (Fig. 5). When *Prochlorococcus* grow at 1 doubling d⁻¹ and growth is balanced by total losses, the ratio of the minimum to maximum would be 0.6, if we assume the cell division occurred only from 19:00 h through midnight. This ratio is inversely related to the length of the time period that *Prochlorococcus* cells are engaged in replication. For example, if cell division started 2 h earlier or later, the ratio of the minimum to maximum of cell concentration would be 0.64 or 0.57, respectively. However, the fact that grazing is probably not constant

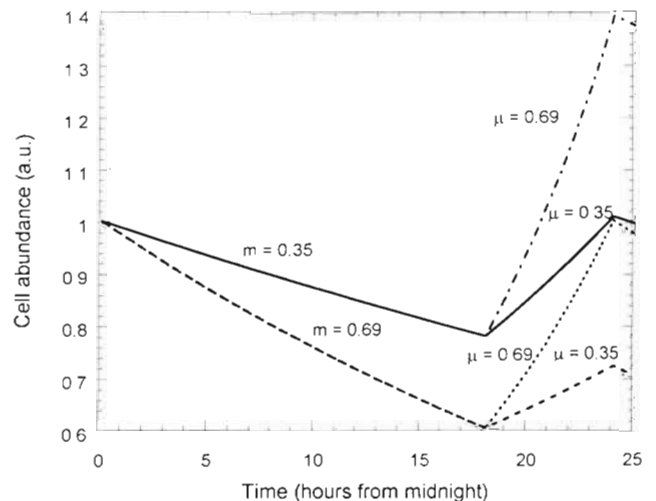


Fig. 5. A simple model for predicting the diel variation in *Prochlorococcus* cell concentrations based on phased cell division (μ is constant from 18:00 h through 24:00 h, and equal to 0 during other times of the day) and constant mortality rates (m is constant throughout the day). The μ (d⁻¹) and m (d⁻¹) shown in the figure are mean growth and mortality rates over a 24 h period. The instantaneous growth rate over the 6 h growth period is actually much higher. The rates of 0.69 and 0.35 d⁻¹ represent 1 and 0.5 doublings d⁻¹, respectively

throughout the day (Christoffersen 1994, Liu H. et al. 1995) makes the diel variation of *Prochlorococcus* abundance less predictable. Assuming no cell division occurred prior to 16:00 h (18:00 h for Station ALOHA), the mortality rates of *Prochlorococcus* in the no-replication period (midnight to 16:00 h for EqPac and 03:00 to 18:00 h for Station ALOHA) were calculated from the decrease in cell concentrations during this period (Fig. 4). *Prochlorococcus* mortality rates during the time period of cell replication were then calculated from the difference between the observed increase in cell number and the determined growth rates (Table 1). Calculated *Prochlorococcus* mortality rates were 0.44, 0.52, 0.37 and 0.45 d^{-1} for the no-division period and 0.90, 0.21, 0.57 and 0.75 d^{-1} during the division period for W-EqPac, E-EqPac, ALOHA Sept 93 and ALOHA July 94, respectively. Thus, it appears that *Prochlorococcus* mortality rate were higher during the time of cell replication, except at the E-EqPac. The mechanisms behind these differences are unclear, but may be related to the feeding rhythms of heterotrophic nanoflagellates (Wikner et al. 1990, Christoffersen 1994). The replication pattern of *Prochlorococcus*, with rapid cell division from dawn through midnight, may lead to increased grazing activity. An alternative explanation may be that rather than changing their grazing activities, phagotrophic protists control the abundance of picoplankton partly by preferentially removing dividing cells (Sherr et al. 1992). Accurate estimates of diel variation in mortality rate require more frequent sampling and depth-specific computation.

Using a cellular carbon content of *Prochlorococcus* of 53 fg C cell⁻¹ (Campbell et al. 1994), we estimated that the daily integrated production rate of *Prochlorococcus* was 409 and 183 mg C m⁻² d⁻¹ at W-EqPac and E-EqPac, respectively (Table 1). The total net primary production at the same stations measured by the ¹⁴C method on the same day of our sampling was 670 and 1330 mg C m⁻² d⁻¹, respectively (data provided by A. Le Bouteiller). This converts to 1039 and 2062 mg C m⁻² d⁻¹ of gross carbon production using the conversion factor of 1.55 determined by Bender et al. (1992). Since our estimation of *Prochlorococcus* production rate is closer to gross production (Liu H. et al. 1995, Vaultot et al. 1995), we conclude that *Prochlorococcus* accounted for up to 39% of total gross primary production at the oligotrophic W-EqPac Station, but only 9% at the relatively nutrient-rich E-EqPac. The contribution of *Prochlorococcus* to total gross primary production at Station ALOHA was much higher due to its extremely high abundance. The depth-integrated concentrations of *Prochlorococcus* at Station ALOHA were more than 2 orders of magnitude higher than those of *Synechococcus* and picoeucaryotes (Campbell & Vaultot

1993). We calculate that *Prochlorococcus* accounted for 74% of total gross primary production in September 1993 and 82% in July 1994. This agrees with the percentage of chlorophyll and carbon biomass represented by *Prochlorococcus* at Station ALOHA (Letelier et al. 1993, Campbell et al. 1994).

Primary production measured with the ¹⁴C method actually represents something between net and gross carbon production (Bender et al. 1987, Grande et al. 1989, Kiddon et al. 1995, Langdon et al. 1995). Bender et al. (1992) scaled the difference between ¹⁴C production and gross carbon production through 2 different approaches. We chose to use the factor determined from the relationship between dawn-to-dusk ¹⁴C production and ¹⁸O production rather than the one determined from estimating ¹⁴C loss from the difference between dawn-to-dusk and 24 h ¹⁴C productivities because we believe that the loss of fixed carbon to DOC or to small particles not retained during filtration is significant and the respiration rate may be greater during the daytime as pointed out by Bender et al. (1992). The average loss of carbon during the night was 21% of the daytime production in the ¹⁴C measurement during the FLUPAC cruise (A. Le Bouteiller pers. comm.). This closely matches the 20% average obtained by Bender et al. (1992) in the North Atlantic Ocean and suggests that our approximation is suitable. Applying the ratio between net and gross production (0.4; Kiddon et al. 1995) to scale the ¹⁴C production to gross carbon production (e.g. Vaultot et al. 1995) will overestimate the total gross production.

The fact that the contribution of *Prochlorococcus* to gross primary production is highest at Station ALOHA, followed by W-EqPac, and lowest at E-EqPac, leads us to conclude that the contribution of *Prochlorococcus* to the total gross primary production increases as the euphotic zone of the water column becomes more oligotrophic. Evidence to suggest that Station ALOHA in the subtropical North Pacific gyre is more oligotrophic than W-EqPac is based on nutrient and primary production data. First, the nitracline, which is defined as the shallowest depth of first appearance of 0.1 μM NO₂+NO₃, is deeper at Station ALOHA than at W-EqPac (Table 1). Second, the depth-integrated NO₂+NO₃ in the upper 150 m of the water column was 57 $\mu\text{mol cm}^{-2}$ at W-EqPac, but only 2.8 to 5.3 $\mu\text{mol cm}^{-2}$ at Station ALOHA. Third, the depth-integrated chlorophyll concentration and primary production at W-EqPac was higher than that at Station ALOHA (Table 1).

Our study shows that *Prochlorococcus* can grow quite rapidly (up to 1 doubling d⁻¹) under conditions of relatively low nutrients and irradiance. The small size and adaptability of the picoplankton enable it to take up dissolved nutrients much more efficiently than

larger organisms (see Fogg 1995). The high growth coupled with high mortality rates at all sites studied suggests that *Prochlorococcus* growth is not severely nutrient-limited in the oligotrophic environment. Its growth rate may be dependent upon rapid cycling of nutrients by protozoan grazing activity. Grazing of protozoa such as phagotrophic flagellates and ciliates not only maintains the picophytoplankton population at a stable level, but also controls picoplankton growth rate by nutrient feedback (Ferrier & Rassoulzadegan 1991, 1994). Because of the phased cell division and variable yet substantial grazing pressure, the abundance of *Prochlorococcus* could vary up to 2-fold depending on the sampling time. Consideration of this diel variation in abundance, as well as daily variations in cell size (Binder et al. 1996) and chlorophyll content (Vaulot & Marie 1995), is needed in future studies of the dynamics of phytoplankton community structure and primary production.

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