

Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique

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ABSTRACT: A selective metabolic inhibitor method has been developed to estimate growth rates and mortalities due to protozoan grazing of the photoautotrophic prokaryotic picoplankton *Prochlorococcus* and *Synechococcus*. Laboratory and field experiments show that 1 mg ml⁻¹ (final concentration) kanamycin inhibits the growth of *Prochlorococcus* and *Synechococcus* effectively and does not significantly affect protozoan grazing. At Station ALOHA (22° 45' N, 158° W) 100 km north of Oahu, Hawaii, USA, growth rates of *Prochlorococcus* ranged from 0.4 to 0.5 d⁻¹ within the surface mixed layer to about 0.1 d⁻¹ at the bottom of the euphotic zone. *Synechococcus* grew faster, with a daily growth rate of up to 1.0 d⁻¹ in surface waters. Grazing mortalities varied for *Prochlorococcus* and *Synechococcus* from 20 to 116% and 43 to 87% of growth rates, respectively. Growth generally exceeded grazing. Because of its high abundance (up to 2 × 10⁵ cells ml⁻¹ in the upper 100 m), *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the subtropical North Pacific Ocean. At Station ALOHA in October 1993, integrated (0 to 175 m) carbon production due to *Prochlorococcus* was 382.2 mg C m⁻² d⁻¹. In contrast, *Synechococcus* produced only 14.6 mg C m⁻² d⁻¹.

KEY WORDS: Growth · Mortality · *Prochlorococcus* · *Synechococcus* · Selective inhibitor · FLB

INTRODUCTION

Within the last decade, photosynthetic picoplankton (<2 µm cells) have been found to account for more than half of the biomass and primary production in the tropical and subtropical open ocean (Li et al. 1983, Platt et al. 1983, Takahashi & Bienfang 1983, Herbrand et al. 1985). Among them, 2 groups of prokaryotic cells, *Synechococcus* and *Prochlorococcus*, were reported to be the predominant components in this size class. The phycoerythrin-containing unicellular cyanobacteria *Synechococcus* were the first to be studied in detail because they are easily recognized by their distinctive orange fluorescence under the epifluorescence microscope (Johnson & Sieburth 1979, Waterbury et al. 1979, Olson et al. 1990a). Early studies reported that *Synechococcus* contributed up to 95% of total primary production (Glover 1985, Iturriaga & Mitchell 1986, Waterbury et al. 1986, Iturriaga & Marra 1988). However, as we now realize, the pres-

ence of *Prochlorococcus* confounded these estimates. *Prochlorococcus*, a new group of oxyphototrophic marine prokaryotes containing divinyl chlorophylls *a* and *b*, were discovered in the mid-1980s by Chisholm et al. (1988) using flow cytometry. *Prochlorococcus* is now recognized as a major component of the picoplankton in warm oceanic waters of the Atlantic Ocean (Li & Wood 1988, Olson et al. 1990), the Pacific Ocean (Chavez et al. 1991, Campbell & Vaulot 1993), as well as the Mediterranean (Vaulot et al. 1990) and Red Seas (Veldhuis & Kraay 1993). The relative importance of *Prochlorococcus* and *Synechococcus* to the total photosynthetic biomass varies among oceanic regions. While *Synechococcus* contributes only 2% of particulate organic carbon (POC) in the central North Pacific, *Prochlorococcus* contributes up to 30% of POC in the upper 200 m water column (Campbell et al. 1994). In the northern Indian Ocean, *Synechococcus* accounts for up to 40% of the POC (Burkill et al. 1993). However, because of the overlap

in cell size between *Prochlorococcus* and *Synechococcus*, the contribution of either individual group to total primary production cannot be obtained accurately by size-fractionated photosynthetic measurements.

The growth rate of *Synechococcus* has been studied quite intensively (Landry et al. 1984, Campbell & Carpenter 1986a, b, Iturriaga & Mitchell 1986, Iturriaga & Marra 1988, Burkill et al. 1993). In contrast, there are only a few recent reports of the growth rates of *Prochlorococcus* (Goericke & Welschmeyer 1993, Vaultot et al. 1994). There are many indirect and direct methods to estimate a population's growth rate (see review by Furnas 1990). Because of grazing, the change in cell abundance in the water column reveals the net growth rate. The dilution method (Landry & Hassett 1982, Landry et al. 1984) and selective metabolic inhibition (Newell et al. 1983, Fuhrman & McManus 1984) techniques are the 2 most frequently used methods to estimate grazing mortalities and compute absolute growth rates of phytoplankton.

The use of selective metabolic inhibitors in the study of marine phytoplankton was first reported for eukaryotes (Thomas & Dodson 1974). Newell et al. (1983), for example, applied the eukaryotic inhibitors cycloheximide and thiram to estimate grazing rates on bacteria. Fuhrman & McManus (1984) modified this method by using benzylpenicillin as a prokaryotic inhibitor for estimating rates of bacterivory in Long Island Sound (New York, USA). Sherr et al. (1986) compared the eukaryotic inhibitors (cycloheximide + colchicine) and prokaryotic inhibitors (vancomycin + penicillin) in the study of trophic interactions between heterotrophic protozoa and bacterioplankton in estuarine waters. Ampicillin, a prokaryotic inhibitor, has also been used to estimate the growth rate of and grazing pressure on *Synechococcus* (Campbell & Carpenter 1986b). However, none of the above have proved satisfactory for *Prochlorococcus* (M. R. Landry unpubl.). Through a series of laboratory and field tests, we found that kanamycin is an effective growth inhibitor for both *Prochlorococcus* and *Synechococcus*. Kanamycin inhibits protein synthesis by combining with the ribosomes to disrupt the genetic code and induce the production of mutant forms (Pelczar et al. 1977). The formation of faulty membrane-associated proteins renders the membrane leaky, causing the loss of essential small molecules and enhanced uptake of more kanamycin, which is then sufficient to prevent further protein synthesis (Greenwood 1989). The key problem in using the selective metabolic inhibitor approach is the specificity of the inhibitor. Therefore, we advocate the addition of fluorescently labeled bacteria (FLB) as an internal control in all incubations to test this critical assumption. We report preliminary results of the kanamycin method for estimating growth rates and grazing

mortality of *Prochlorococcus* and *Synechococcus* populations.

MATERIALS AND METHODS

Culture. *Prochlorococcus* and *Synechococcus* strains were isolated from Station ALOHA, located about 100 km north of Oahu, Hawaii, USA, in the central Pacific Ocean (22° 45' N, 158° W). *Prochlorococcus* was cultured in PC media (modified K/2) as recommended by the Culture Collection of Marine Plankton at Bigelow Laboratory (West Boothbay Harbor, ME, USA). *Synechococcus* was grown in f/2 media. Neither *Prochlorococcus* nor *Synechococcus* cultures are axenic. All culture containers were carefully cleaned by first soaking in 10% hydrochloric acid (HCl) for at least 24 h. The containers were then rinsed with distilled deionized water (DDW) several times, soaked in DDW for more than 24 h, rinsed several times, and autoclaved at 121°C for 20 min.

Laboratory experiments. Kanamycin stock solutions (10 to 100 mg ml⁻¹) were made immediately prior to use by dissolving kanamycin (Sigma, K-4000) in DDW and filter sterilizing (Acrodisc 0.2 µm syringe filters).

To determine the kanamycin concentration required to inhibit growth without causing cell lysis or death, we performed growth experiments with final kanamycin concentrations of 0, 0.01, 0.10, 0.50 and 1.0 mg ml⁻¹. Triplicate cultures of *Prochlorococcus* and *Synechococcus* were grown in 20 ml glass test tubes under continuous illumination of approximately 20 µEin m⁻² s⁻¹. Samples (0.5 ml) were taken at 6 h intervals from each test tube using sterile technique, preserved with paraformaldehyde (0.2% final concentration), frozen quickly in liquid nitrogen, and stored at -80°C. Thawed samples were analyzed by flow cytometry following the method of Vaultot et al. (1989).

To test whether kanamycin affected the grazing activity of protozoans, 2 enrichment cultures, a heterotrophic nanoflagellate (HNAN) and a mixture of nanoflagellates and ciliates from Kaneohe Bay (KEW), were grown in rice culture media with 0, 0.1 and 1.0 mg ml⁻¹ kanamycin. FLB were made from *Vibrio damsela* with 5-[(4,6-dichlorotriazin-2-yl)amino]-fluorescein (DTAF) following the procedure of Sherr et al. (1987), and were added to triplicate experimental tubes at a final concentration of 20 000 ml⁻¹. Samples (0.5 ml) were taken every 6 h for flow cytometric determination of FLB abundance. Grazing rates were computed from the rate of decline of FLB concentration with time.

Field experiments. Field experiments were conducted in Kaneohe Bay, Hawaii, during February 1993 and at the Hawaii Ocean Time-series (HOT) Station ALOHA in

May, September and October 1993. As described below, some details varied among experiments. Similarities included incubations of triplicate 250 ml polycarbonate bottles for each treatment and control. In addition, FLB were added to all bottles at a final concentration of 10 000 cells ml⁻¹ to test whether the kanamycin inhibited grazing activity and/or to correct for an inhibitor effect on grazing if it occurred. For individual experiments, we used the difference between FLB disappearance rates in the incubation treatments and controls to compute inhibitor-corrected growth and grazing rates for *Prochlorococcus* and *Synechococcus*. In most cases, we observed a small effect of kanamycin on grazing activity based on the difference in FLB disappearance rates between treatments. The difference was most often statistically insignificant due to a large standard deviation and small sample number (see Table 1). We did, however, correct our grazing and growth rates because of the apparent effect.

Initial experiments in Kaneohe Bay were conducted to verify the effective kanamycin concentration for natural populations. Seawater samples were collected before dawn from the sea surface inside and from adjacent oceanic waters outside the bay. Kanamycin was added to the bottles to yield final concentrations of 0 (control), 0.01, 0.1 and 1.0 mg ml⁻¹. All bottles were incubated *in situ* in surface waters near Coconut Island in Kaneohe Bay for 24 h. A 1 ml subsample was taken from each bottle every 6 h using the same procedure described for laboratory experiments.

For experiments at Station ALOHA, Go-Flo bottles were used to collect seawater before sunrise from 5, 45 and 100 m in May and 75 and 125 m in September. Based on results from Kaneohe Bay, only 1 kanamycin concentration (1 mg ml⁻¹) and a control were prepared. Triplicate bottles for the treatment and control were incubated in a shipboard temperature- and light-controlled incubator, and an additional set of triplicate samples was incubated *in situ* in order to compare the 2 incubation techniques. The *in situ* incubation lasted between dawn and dusk, and subsamples for flow cytometric analyses were taken at the beginning and end of the experiment. Subsamples were taken every 6 h during the 24 h shipboard incubations.

For the October experiment, seawater was collected from 8 depths from 5 to 175 m. The incubation procedure was also modified because previous results revealed that cell division of *Prochlorococcus* occurred more during nighttime and, therefore, that dawn-to-dusk incubations underestimated specific growth rates. Triplicate bottles were incubated *in situ* from dawn-to-dusk followed by shipboard incubation after dark to complete a 24 h incubation.

Flow cytometry. All samples were analyzed using a Coulter EPICS 753 flow cytometer equipped with two

5 W Argon lasers and MSDS automatic sampling. Filter set-ups were as previously described by Campbell & Vaulot (1993). For *Prochlorococcus* and *Synechococcus*, we used an excitation wavelength of 488 nm (1 W) to collect green (525 nm ± 40 df), orange (575 nm ± 40 df) and red (680 nm ± 40 df) fluorescence and right angle light scatter (RALS), which varies as a function of cell size. Sample volumes were 100 µl for most analyses, however, 200 to 300 µl were analyzed for some samples to obtain a statistically significant cell number for *Synechococcus*. *Prochlorococcus* and *Synechococcus* populations were distinguished from heterotrophic bacteria by their respective red (divinyl chlorophyll) and orange (phycobilin) autofluorescences under 488 nm excitation. FLB were characterized by bright green fluorescence. In laboratory experiments, subsamples for enumeration of heterotrophic bacteria were stained with 1 µg ml⁻¹ Hoechst 33342 (Molecular Probes, Eugene, OR, USA) and analyzed with dual UV/488 nm excitation (Monger & Landry 1993). Heterotrophic bacteria have blue fluorescence under UV excitation (DNA content) but lack any autofluorescence. List mode files were analyzed on an IBM PC-compatible microcomputer using CytoPC software (Vaulot 1989).

Calculations. Instantaneous rates of population growth and grazing mortality were calculated for *Prochlorococcus* and *Synechococcus* as described by Campbell & Carpenter (1986b). Protozoan grazing on FLB in all experiments were determined from the same exponential model. Integrated growth rates (d⁻¹) in the upper 175 m water column are calculated from:

$$\mu_{\text{int}} = \ln \left[\frac{\int N_{24}(z) dz}{\int N_0(z) dz} \right]$$

where N_0 is cell abundance at the beginning time of incubation, N_{24} is cell abundance after the 24 h incubation without accounting for grazing (i.e. $N_{24} = N_0 e^{\mu}$), and z is the sampling depth. For the experiments at Station ALOHA, carbon production of *Prochlorococcus* and *Synechococcus* were calculated from cell abundance, growth rates and a carbon/cell conversion factor and compared with total primary production measured by ¹⁴C method (data provided by Joint Global Ocean Flux Study). We assumed conversion factors of 53 and 250 fg C cell⁻¹ for *Prochlorococcus* and *Synechococcus*, respectively (Campbell et al. 1994). Carbon production (P , mg C m⁻³ d⁻¹) at each sampling depth was calculated as:

$$P = N \times \text{fg C cell}^{-1} \times 10^{-6}$$

where N (cell ml⁻¹ d⁻¹) is the number of cells produced in 24 h which can be computed from

$$N = N_0 (e^{\mu} - 1).$$

RESULTS

Laboratory experiments

Laboratory incubations with different concentrations of kanamycin indicated that concentrations of 1 and 0.5 mg ml⁻¹ prevented cell division of *Prochlorococcus* and *Synechococcus*, respectively (Fig. 1). Kanamycin had no effect on the growth of heterotrophic bacteria co-occurring in the cultures of laboratory experiments. Grazing experiments showed no statistically significant difference in protozoan grazing on FLB between the control and kanamycin treatments (Fig. 2) (ANOVA: $F = 2.13$, $p = 0.234$, $df = 2$ for HNAN; $F = 0.86$, $p = 0.469$, $df = 2$ for KEW). We chose a kanamycin concentration of 1 mg ml⁻¹ for all subsequent field experiments.

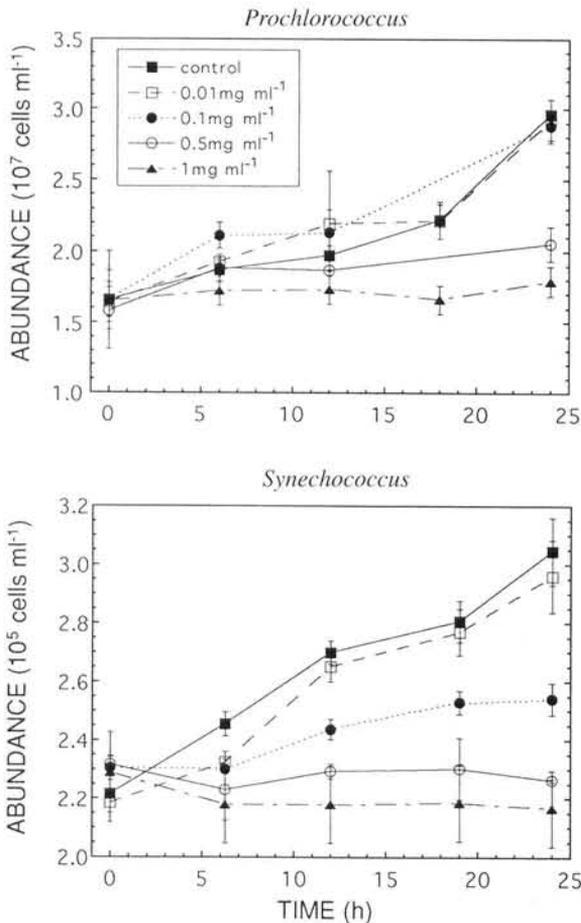


Fig. 1. *Prochlorococcus* (Pacific strain) and *Synechococcus*. Laboratory experiments with different concentrations of kanamycin under continuous light. For *Synechococcus*, curves of control and 0.5 mg kanamycin ml⁻¹ (final concentration) are significantly different (t -test, $p < 0.005$). For *Prochlorococcus*, 1 mg kanamycin ml⁻¹ prevents cell division (t -test, $p < 0.0025$). Error bars show 1 SD of the means of triplicate incubations

Kaneohe Bay field studies

The results of the Kaneohe Bay experiments showed a slight decrease of protozoan grazing on FLB in samples with 1 mg ml⁻¹ of kanamycin, but no significant difference from controls (Table 1, Fig. 3b, c). The slower decrease of *Prochlorococcus* abundance in the 1 mg ml⁻¹ kanamycin incubations than in other treatments also suggests that protozoan grazing activity was affected, in particular during the first few hours after adding kanamycin to incubations (Fig. 3a). However, this grazing inhibition during the first 6 h was not observed clearly for *Synechococcus* (Fig. 3d, e).

The growth rates of *Synechococcus* measured in Kaneohe Bay and in oceanic waters were similar, 0.70 and 0.58 d⁻¹, respectively. *Prochlorococcus* growth rate was substantially lower, 0.33 d⁻¹ for oceanic waters. *Prochlorococcus* were not observed inside Kaneohe Bay. The daily grazing mortality of *Prochlorococcus* and *Synechococcus* calculated from 24 h *in situ* incubations revealed that only about 30% of the pro-

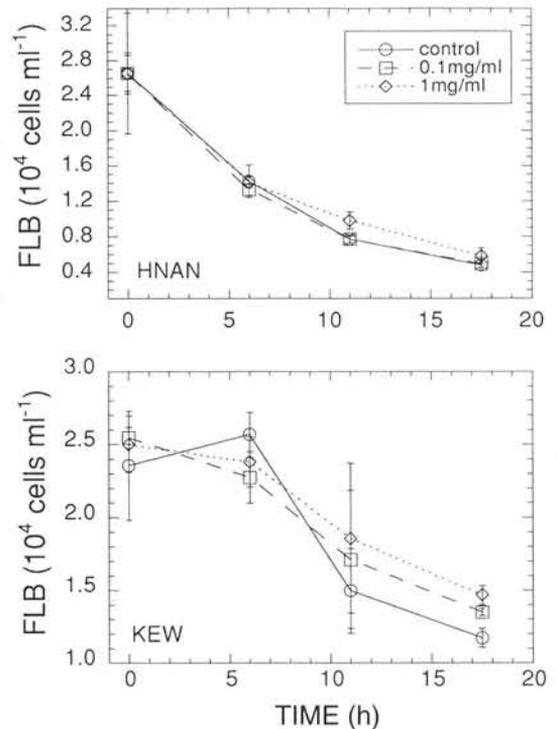


Fig. 2. Results of laboratory experiments to test the effect of kanamycin on protozoan grazing. Data show no difference of protozoan grazing on fluorescently labeled bacteria (FLB) between treatments. HNAN: a culture of a heterotrophic nanoflagellate (ANOVA, $F = 2.13$, $p = 0.234$, $df = 2$); KEW: a mixture of nanoflagellates and ciliates (ANOVA, $F = 0.86$, $p = 0.469$, $df = 2$). Error bars show 1 SD of the means of triplicate incubations

Table 1. The difference in protozoan grazing on FLB in the control and inhibitor (1 mg ml⁻¹ kanamycin) incubations tested by *t*-test. Data are the means of triplicate measurements \pm 1 SD calculated from 24 h incubations. In the May 1993 experiment, rates from dawn-to-dusk incubation are given in parentheses. * *p* < 0.05; ns: *p* > 0.05

Location Depth	Grazing rates on FLB (d ⁻¹)		<i>t</i> -test
	Control	Inhibitor	
Kaneohe Bay			
Bay, surface	0.47 \pm 0.07	0.35 \pm 0.10	1.70 ns
Oceanic, surface	0.51 \pm 0.06	0.39 \pm 0.07	2.25 ns
Station ALOHA			
May 1993			
5 m	0.30 \pm 0.10 (0.62 \pm 0.10)	0.27 \pm 0.10 (0.60 \pm 0.01)	0.37 ns (0.34) ns
45 m	0.38 \pm 0.03 (0.67 \pm 0.05)	0.29 \pm 0.14 (0.50 \pm 0.28)	1.09 ns (1.04) ns
100 m	0.30 \pm 0.07 (0.17 \pm 0.02)	0.32 \pm 0.07 0.08 \pm 0.00	0.35 ns (6.33)*
October 1993			
5 m	0.49 \pm 0.14	0.29 \pm 0.24	1.25 ns
25 m	0.33 \pm 0.02	0.26 \pm 0.10	1.19 ns
45 m	0.17 \pm 0.13	0.14 \pm 0.04	0.38 ns
75 m	0.10 \pm 0.07	0.13 \pm 0.02	0.71 ns
100 m	0.20 \pm 0.09	0.18 \pm 0.14	0.21 ns
125 m	0.21 \pm 0.08	0.12 \pm 0.10	1.22 ns
150 m	0.08 \pm 0.04	0.09 \pm 0.05	0.27 ns
175 m	0.13 \pm 0.04	0.09 \pm 0.11	0.59 ns
Overall (n = 40)	0.32 \pm 0.18	0.25 \pm 0.18	1.66 ns

duction of *Prochlorococcus* in the surface water was lost to grazing. In comparison, the grazing pressure on *Synechococcus* spp. at both sites was 38 to 56% of its daily growth (Table 2).

Station ALOHA field studies

Results from 24 h deck incubations during the first experiment (May 1993; Figs. 4 to 6) indicated that *Prochlorococcus* grows slowly under oligotrophic conditions. Calculated growth rates averaged 0.20 (\pm 0.03) d⁻¹ at 45 m and 0.10 (\pm 0.09) d⁻¹ at 100 m. Growth rates of *Prochlorococcus* in the September experiments were similar with higher rates measured at 125 m (0.17 \pm 0.06 d⁻¹) than at 75 m (0.09 \pm 0.01 d⁻¹). The growth rates of *Prochlorococcus* calculated from dawn-to-dusk (i.e. 12 to 14 h) *in situ* incubations in these experiments were lower (0.09 \pm 0.06 d⁻¹ at 45 m and 0.04 \pm 0.01 d⁻¹ at 100 m in May, and 0.05 \pm

0.05 d⁻¹ at 75 m in September) because cell division of *Prochlorococcus* occurs at a higher rate during the night (Fig. 5). In October, a higher *Prochlorococcus* growth rate of 0.59 d⁻¹ (SE = 0.09) was found at 25 m. Again, the growth rates of *Prochlorococcus* were lowest at 75 m (0.09 d⁻¹) and increased slightly below (Table 2, Fig. 7).

Synechococcus grew faster than *Prochlorococcus*, with growth rates ranging from 0.39 to 0.70 d⁻¹ throughout the upper 100 m water column during May and September (Table 2). In contrast, during October *Synechococcus* growth rate was much higher (1.06 d⁻¹, SE = 0.09) near the sea surface but decreased to 0.17 d⁻¹ (SE = 0.04) at 75 m (Table 2, Fig. 7). The integrated growth rates in the upper 175 m water column are 0.26 d⁻¹ for *Prochlorococcus* and 0.44 d⁻¹ for *Synechococcus*.

Mortality rates of *Prochlorococcus* measured in 24 h incubations at Station ALOHA ranged from 0.04 to 0.50 d⁻¹ within the upper 175 m water column (Table 2). The ratio of grazing to growth estimates ranged from 0.20 to 1.16. Grazing on *Synechococcus* seemed less variable with grazing to growth ratios of 0.43 to 0.87. Grazing mortality (especially for *Prochlorococcus*), measured in *in situ* dawn-to-dusk incubations, was higher than in 24 h incubations and always resulted in negative net growth rates for *Prochlorococcus*. Grazing of FLB showed the same pattern

Table 2. *Prochlorococcus* and *Synechococcus*. Grazing rates (*g*) and growth coefficients (μ) in Kaneohe Bay and at Station ALOHA measured by selective inhibitor method. Data are the means of triplicate 24 h incubations \pm 1 SD

Location Depth	<i>Prochlorococcus</i>		<i>Synechococcus</i>	
	μ (d ⁻¹)	<i>g</i> (d ⁻¹)	μ (d ⁻¹)	<i>g</i> (d ⁻¹)
Kaneohe Bay				
Inside, surface			0.70 \pm 0.04	0.39 \pm 0.05
Outside, surface	0.33 \pm 0.02	0.10 \pm 0.02	0.58 \pm 0.21	0.22 \pm 0.07
Station ALOHA				
May 1993				
5 m			0.54 \pm 0.08	0.29 \pm 0.14
45 m	0.20 \pm 0.03	0.04 \pm 0.02	0.44 \pm 0.11	0.26 \pm 0.10
100 m	0.10 \pm 0.09	0.09 \pm 0.06	0.58 \pm 0.09	0.46 \pm 0.01
September 1993				
75 m	0.09 \pm 0.01	0.10 \pm 0.06	0.39 \pm 0.22	0.27 \pm 0.02
125 m	0.17 \pm 0.06	0.12 \pm 0.08		
October 1993				
5 m	0.38 \pm 0.10	0.44 \pm 0.01	1.06 \pm 0.18	0.73
25 m	0.59 \pm 0.19	0.50 \pm 0.16	0.45 \pm 0.30	0.39 \pm 0.18
45 m	0.10 \pm 0.03	0.06 \pm 0.03	0.67 \pm 0.31	0.47 \pm 0.06
75 m	0.09 \pm 0.02	0.05 \pm 0.06	0.17 \pm 0.13	0.09 \pm 0.08
100 m	0.15 \pm 0.14	0.14 \pm 0.18		
125 m	0.13 \pm 0.05	0.14 \pm 0.07		
150 m	0.11 \pm 0.10	0.06 \pm 0.02		
175 m	0.21 \pm 0.11	0.14 \pm 0.02		

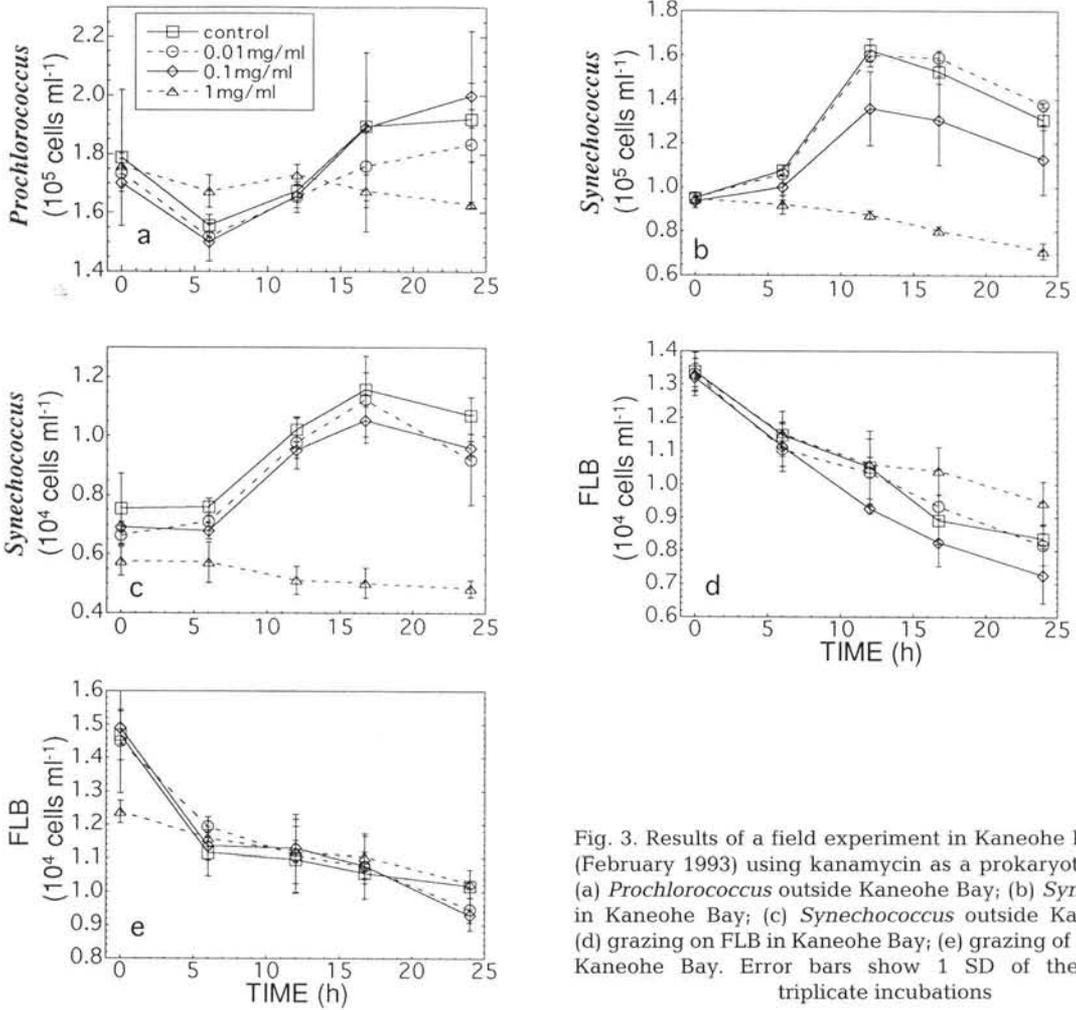


Fig. 3. Results of a field experiment in Kaneohe Bay, Hawaii (February 1993) using kanamycin as a prokaryotic inhibitor. (a) *Prochlorococcus* outside Kaneohe Bay; (b) *Synechococcus* in Kaneohe Bay; (c) *Synechococcus* outside Kaneohe Bay; (d) grazing on FLB in Kaneohe Bay; (e) grazing of FLB outside Kaneohe Bay. Error bars show 1 SD of the means of triplicate incubations

which probably implies higher grazing activities of protozoans during the daylight hours (Table 2). Overall, the growth rates and grazing mortalities of both *Prochlorococcus* and *Synechococcus* showed the similar trend with depth, but growth exceeded grazing in most cases (Table 2, Fig. 7).

An alternative way of estimating the contribution of both groups to total primary production is to determine specific growth rates of *Prochlorococcus* and *Synechococcus* and convert to carbon production with carbon/cell conversion factors. The calculated carbon production of *Prochlorococcus* at Station ALOHA ranged from 10.05 mg C m⁻³ d⁻¹ in surface water to 0.04 mg C m⁻³ d⁻¹ at the bottom of the euphotic zone (Table 3, Fig. 7). It is interesting to note that *Prochlorococcus* contributed the most to production in the surface waters and at 125 m (Table 3). *Synechococcus* was only detected in the upper 100 m of the water column and accounted for less than 5% of the total primary production (Table 3, Fig. 7b).

DISCUSSION

Selective metabolic inhibitors have several advantages for estimating the growth rates of the marine photosynthetic picoplankton *Prochlorococcus* and *Synechococcus* and their mortality due to grazing activities of heterotrophic nanoplankton. These include minimal manipulation of seawater samples, no radioisotopes, and the ability to measure simultaneously growth and grazing for natural bacterial plankton populations (Campbell & Carpenter 1986b, Sherr et al. 1986). The application of the specific inhibitor methods depends on both the effectiveness and specificity of the inhibitor. In our experiments, 1 mg ml⁻¹ kanamycin successfully inhibits cell division of *Prochlorococcus* and *Synechococcus*. However, it also seems to exert a minor effect on the grazing activity of protozoans (see Table 2). Although this effect was not statistically significant in our experiments, it is essential to include the FLB control in all grazing experi-

ments. One possible impact of the inhibition of protozoan activity is a decrease in growth rates of *Prochlorococcus* and *Synechococcus* due to a decrease in the availability of remineralized nitrogen or phosphorus during the incubations. Another potential artifact is an alteration of the physiological status of prokaryotic picoplankton. We found that the chlorophyll fluorescence of *Prochlorococcus* in the surface mixed layer samples in some cases decreased after incubation with the addition of kanamycin. This may have been due to an effect of kanamycin on chlorophyll synthesis or turnover rate.

Our results suggest that a 24 h incubation period is critical for obtaining an unbiased growth rate because of diel cycles of DNA synthesis and growth in *Synechococcus* (Campbell & Carpenter 1986a, Waterbury et

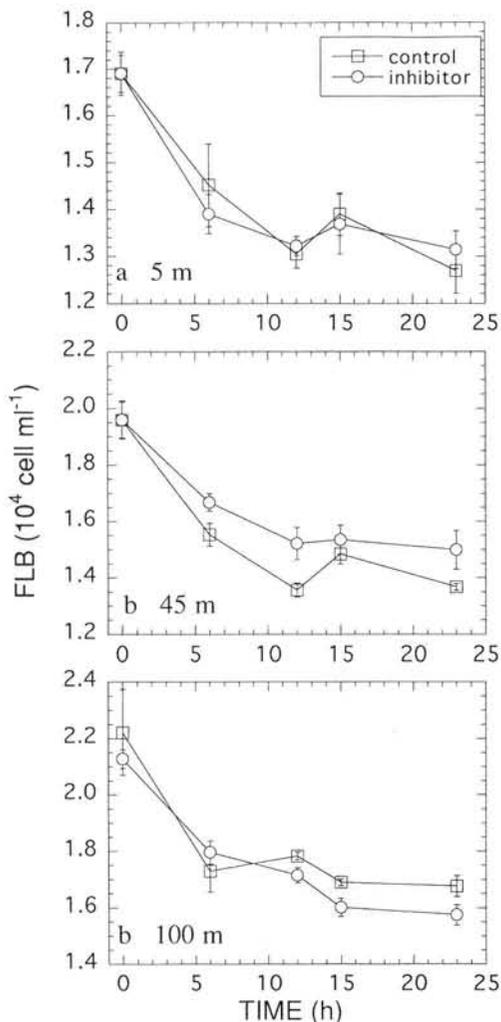


Fig. 4. Changes in the density of FLB in 24 h shipboard incubations at Station ALOHA in May 1993. Error bars show 1 SE of the means of triplicate incubations. Control: natural seawater; inhibitor: 1 mg ml^{-1} kanamycin (final concentration)

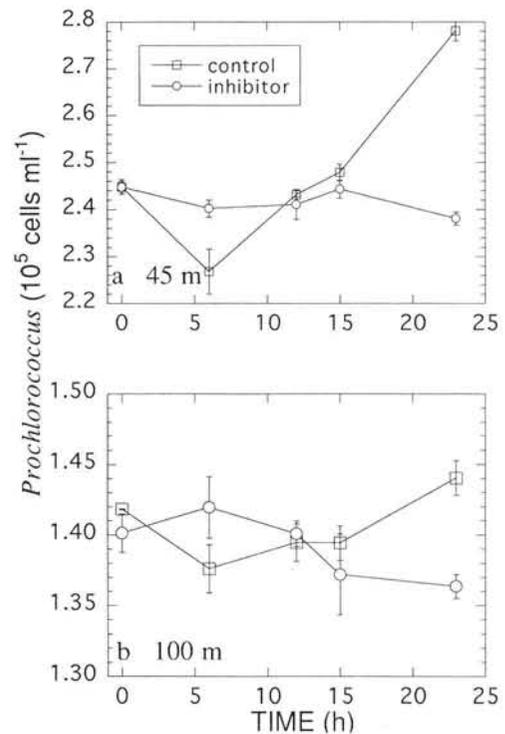


Fig. 5. *Prochlorococcus*. Changes in density in 24 h shipboard incubations at Station ALOHA in May 1993. Error bars show 1 SE of the means of triplicate incubations. Control: natural seawater; inhibitor: 1 mg ml^{-1} kanamycin

al. 1986, Armbrust et al. 1989) and *Prochlorococcus* (Vaulot et al. 1994). The highest division rates of *Synechococcus* were realized in early evening, i.e. 12 to 15 h after dawn (Campbell & Carpenter 1986a), whereas *Prochlorococcus* started cell division after sunset (Vaulot et al. 1994). Therefore, dawn-to-dusk incubations underestimate the 'true' daily growth rate, especially for *Prochlorococcus*.

Our results show that average daily growth rates for *Prochlorococcus* range from 0.4 to 0.6 d^{-1} in near-surface waters and 0.1 to 0.2 d^{-1} at the base of the euphotic zone. Our estimates are close to the observations of Goericke & Welschmeyer (1993) for *Prochlorococcus* in the Sargasso Sea near Bermuda using the ^{14}C labeling technique. However, they are lower than the observation of Vaulot et al. (1994) for the equatorial Pacific (0.52 to 0.65 d^{-1} integrated for upper 150 m) based on cell cycle analysis (Carpenter & Chang 1988). The growth rate of *Synechococcus* measured in Kaneohe Bay (0.58 to 0.70 d^{-1}) and at Station ALOHA (0.17 to 1.06 d^{-1}) are lower than those measured in previous studies in the same bay (Landry et al. 1984) and in the oligotrophic North Pacific (Iturriaga & Mitchell 1986), but similar to the rates measured in the northwest Atlantic Ocean (Campbell & Carpenter 1986b) and the northwest Indian Ocean (Burkill et al. 1993).

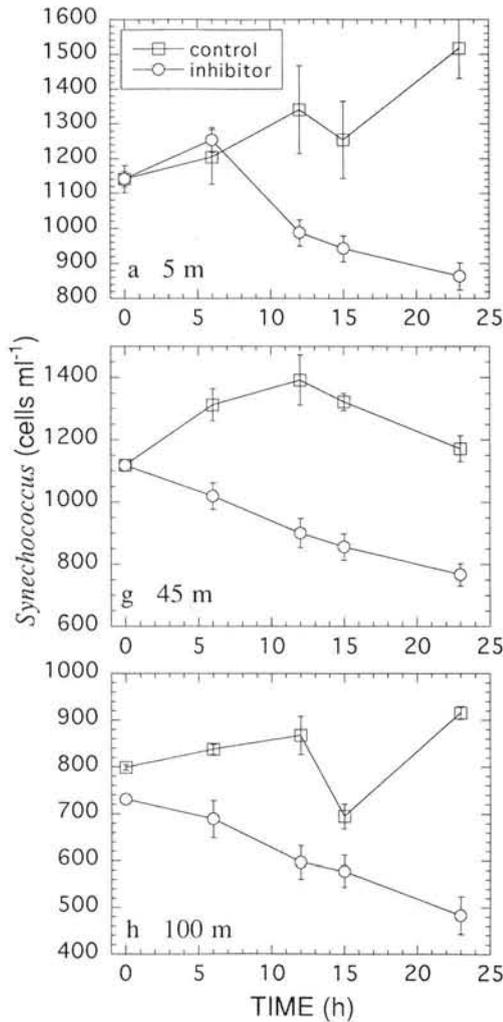


Fig. 6. *Synechococcus*. Changes in density in 24 h shipboard incubations at Station ALOHA in May 1993. Error bars show 1 SE of the means of triplicate incubations. Control: natural seawater; inhibitor: 1 mg ml⁻¹ kanamycin

The trophic interactions between heterotrophic nanoplankton and bacterioplankton in the open ocean are one of the key links of the microbial loop (Azam et al. 1983). Heterotrophic flagellates and ciliates are thought to be the major grazers of picoplankton, including heterotrophic bacteria, *Prochlorococcus*, *Synechococcus* and picoeukaryotes (Sieburth 1984). Our estimates of grazing pressure on *Synechococcus* in Kaneohe Bay (0.22 to 0.39 d⁻¹) and at Station ALOHA (0.09 to 0.73 d⁻¹) are in close agreement with previous reports (Landry et al. 1984, Campbell & Carpenter 1986b, Iturriaga & Mitchell 1986). The estimates of grazing mortality on *Synechococcus* ranged from 43 to 87% of growth rate. The grazing rate on *Prochlorococcus* observed outside Kaneohe Bay and in 24 h incubations at Station ALOHA are more variable (20 to

>100% of the estimated growth rates). The parity of growth and grazing rates for both *Prochlorococcus* and *Synechococcus* indicates that they are significant components of the marine pelagic food web and that most of their biomass is recycled fairly rapidly within the euphotic zone. Standard productivity experiments, such as post-incubation size-fractionated ¹⁴C experiments, may underestimate production of picoplankton because heterotrophic nanoplankton are not eliminated by pre-screening samples before incubation. Also, calculating carbon production from net growth rate (growth - grazing; Veldhuis et al. 1993) will greatly underestimate the production of *Prochlorococcus* and *Synechococcus*.

One interesting phenomenon is the negative growth rate of *Prochlorococcus* observed in most dawn-to-dusk incubations. This trend could be explained by either a diel cycle in cell growth or heavier grazing during daytime. Our data show that grazing on FLB is more intense in dawn-to-dusk *in situ* incubations than in the 24 h shipboard incubation (Table 1). The disappearance of FLB in 24 h incubations (Fig. 4) was also faster during daytime. Whether this is attributable to the existence of a diel cycle in grazing activity or an increase in the abundance of heterotrophic nanoplankton needs further investigation. Based on the diel cycle of growth and grazing of *Prochlorococcus* and

Table 3. *Prochlorococcus* and *Synechococcus*. Total primary production and the production contributed in the upper 175 m water column at Station ALOHA. Total primary production as measured by the ¹⁴C uptake method; data courtesy of Tupas et al. (1994). Production values of *Prochlorococcus* and *Synechococcus* are calculated from growth rate measurements (see text for details). Integrated: from surface to 175 m (October 1993 only) (mg C m⁻² d⁻¹)

Depth	Carbon production (mg C m ⁻³ d ⁻¹)		
	Total	<i>Prochlorococcus</i>	<i>Synechococcus</i>
May 1993			
5 m	9.07		0.20
45 m	8.25	2.87	0.16
100 m	3.05	0.78	0.15
September 1993			
75 m	3.81	1.05	0.19
125 m	0.60	0.64	
October 1993			
5 m	4.61	5.88	0.29
25 m	3.29	10.05	0.15
45 m	2.22	1.36	0.18
75 m	1.51	1.25	0.09
100 m	1.16	0.62	0.01
125 m	0.29	0.27	
150 m	0.11	0.06	
175 m	0.05	0.04	
Integrated	274	382.2	14.6

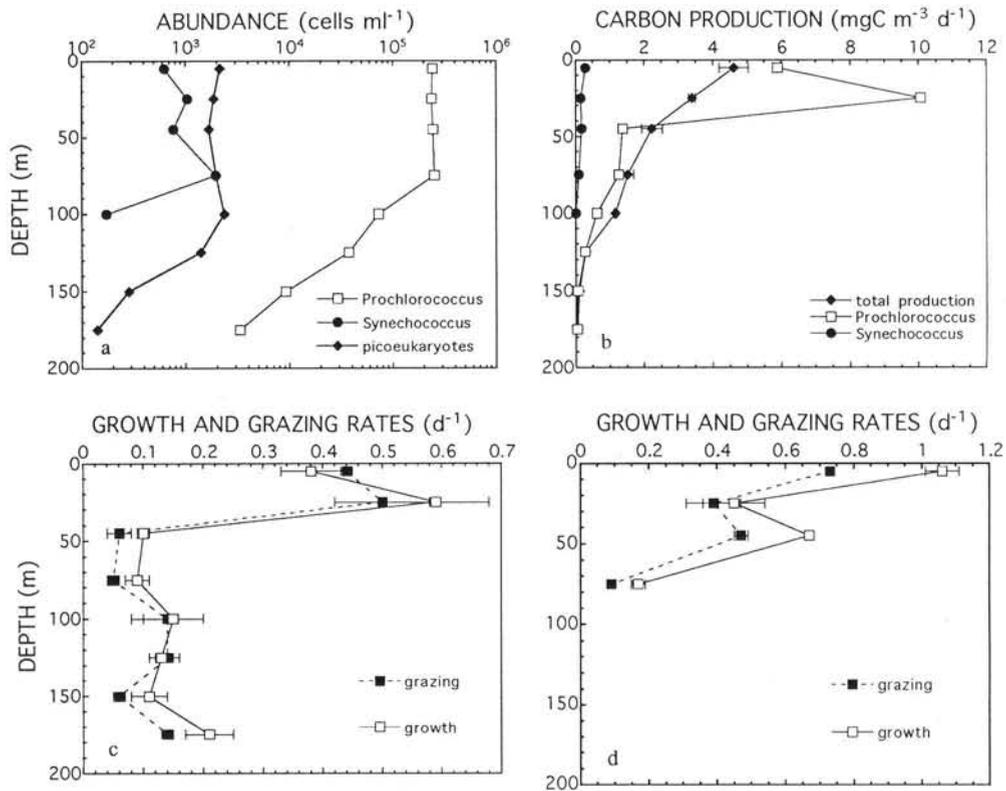


Fig. 7. Depth profile of upper 200 m water column at Station ALOHA in October 1993. (a) Vertical distribution of *Prochlorococcus*, *Synechococcus* and piceoekaryotes. Data are the means of samples at the start point. (b) Carbon production at different depths; total production was measured by the ¹⁴C method (see Tupas et al. 1994) and production of *Prochlorococcus* and *Synechococcus* is calculated from growth rates (Table 2). (c) Growth rates and grazing mortalities of *Prochlorococcus* measured by selective inhibitor method in dawn-to-dusk *in situ* incubations followed by dark deck incubations (means \pm SE). (d) Growth and grazing rates of *Synechococcus* measured in the same experiment

Synechococcus, dawn-to-dusk *in situ* incubations are inappropriate for estimating daily growth and grazing rates. Dawn-to-dusk *in situ* incubations followed by shipboard incubations during the dark period gave reasonable results in our October experiments at Station ALOHA.

Prochlorococcus is extremely important at Station ALOHA in terms of cell abundance and carbon biomass in comparison to the Atlantic and Indian Oceans. On the average, *Prochlorococcus* spp. represent 64% of the total photosynthetic carbon biomass in the upper 200 m at Station ALOHA (Campbell et al. 1994). The percentages of *Prochlorococcus* to the total photosynthetic carbon biomass in the surface mixed layer, deep chlorophyll maximum, and $<0.05\%$ I_0 (generally 150 to 170 m) are 73%, 47% and 33%, respectively. Our data show that *Prochlorococcus* also contributes significantly to primary production at Station ALOHA, but there is temporal variation between cruises (Table 3, Fig. 7). We cannot calculate the exact percentages of *Prochlorococcus* and *Synechococcus* contributions to total primary production because our estimations are

not comparable to the total primary production measured by ¹⁴C method. Our calculation is closer to the gross production, whereas the ¹⁴C method does not include carbon uptake that is subsequently ingested by protozoa and lost to respiration and DOC release. In October 1993, the integrated primary production due to *Prochlorococcus* in the upper 175 m at Station ALOHA was 382.2 mg C m⁻² d⁻¹, which exceeds the integrated total primary production measured by the ¹⁴C method (274 mg C m⁻² d⁻¹). Nevertheless, this result indicates that *Prochlorococcus* contribute significantly to primary production in the subtropical North Pacific Ocean. Given its relatively low contribution to community biomass (Campbell et al. 1994), *Synechococcus* does not significantly contribute to the total primary production at Station ALOHA (Table 3).

The carbon/cell conversion factor may also affect the accuracy of the estimation of primary production. The conversion factors we used in this paper were based on 0.47 pg C μm^{-3} from Verity et al. (1992) which is about 4 times higher than those used in earlier studies (Glover et al. 1988). Also, the size of *Prochlorococcus*

cells is smaller in near-surface waters and increases with depth (Campbell et al. 1994), as does *Synechococcus* (Olson et al. 1990b, Burkill et al. 1993).

The contribution of *Prochlorococcus* to total primary production is higher in surface water. At the chlorophyll maximum layer, which varies between 100 and 120 m, the contribution from picoeukaryotes becomes more important as shown in cell abundance (Fig. 7a) and biomass (Campbell et al. 1994). Our method is inappropriate for eukaryotic algae. Results from dilution experiments at Station ALOHA showed that the algae grew faster than *Prochlorococcus* and *Synechococcus* (data not shown) and they should certainly contribute significantly more to primary production than *Synechococcus*.

Because *Prochlorococcus* is a dominant component of the phytoplankton community in the tropical and subtropical open-ocean ecosystems, understanding its contribution to photosynthetic biomass and primary production in the global oceans is relevant to elucidating the dynamics of pelagic marine food webs. The implications of these results are important with regard to issues such as global ocean flux. We have demonstrated the effectiveness of kanamycin use in the selective inhibitor technique. In using this approach together with flow cytometric enumeration, we estimate that the contribution of *Prochlorococcus* to primary production in the North Pacific is approximately 1 order of magnitude greater than that of *Synechococcus*.

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