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Estimating *Synechococcus* spp. growth rates and grazing pressure by heterotrophic nanoplankton in the English Channel and the Celtic Sea

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Abstract ——Marine chroococcoid phycoerythrin - containing *Synechococcus* spp. recently have been implicated as a substantial component of the photosynthetic picoplankton in the ocean. Although the importance of *Synechococcus* as food sources for heterotrophic nanoplankton are now recognized, the information about its cycling of biomass and diel patterns is limited and the methodology used varies according to different authors. A selective metabolic inhibitor method was used to allow simultanous estimation of both growth rates and grazing disappearance rates of *Synechococcus*. Results obtained in the English Channel show growth rates ranging from 0. 25 to 0. 72 d^{-1} with an average value of 0. 51 d^{-1} and grazing disappearance rates ranged from 0. 21 to 0. 64 d^{-1} (mean = 0. 44 d^{-1}). Offshore in the Celtic Sea of the Northeast Atlantic Ocean, both rates were lower than in the channel. The similarity between average growth and grazing rates suggests a rapid recycling of *Synechococcus* biomass. In diel pattern, *Synechococcus* grazing mortality rates were higher during the day (mean = 0. 61 d^{-1}) than during the night (mean = 0. 21d^{-1}) in all the experiments. A positive correlation was observed between growth rates and *in situ* temperature ranged from 9 to 20℃. Size-fractionated experiments demonstrate that up to about 70% of *Synechococcus* disappearance could be attributed to the grazer smaller than 2 μm in diameter. The variations of *Synechococcus* cell characteristics such as size and phycoerythrin contents in the growth and grazing experiments were determined with a flow cytometer. The methodology recently used on estimating dynamics of *Synechococcus* population is reviewed.

INTRODUCTION

The presence of chroococcoid cyanobacteria of the genus *Synechococcus* spp. in the marine environment was discovered a few years ago (Waterbury *et al.*, 1979; Johnson and Sieburth, 1979), but only recently these organisms have been recognized well as a substantial component of the autotrophic picoplankton in most regions of the world's oceans (Platt *et al.*, 1983; Takahashi and Bienfang, 1983; Li *et al.*, 1983; Glover, 1985). In consequence the tranditional pattern of a netplankton-copepod-fish pelagic food chain has to be revised (Azam *et al.*, 1983). Sieburty and Davis (1982) pointed out that cyanobacteria are abundant enough to be an essential food source for heterotrophic

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nanoplankton be-cause the biomass of the former is of about one order of magnitude higher than that of the latter. As the importance of the numerically dominant picoplankton in the photosynthetic production (Glover *et al.*, 1986) and the pelagic food web dynamics (Iturriaga and Mitchell, 1986) has been recognized, the cycling of *Synechococcus* biomass plays a dominant role in the trophic relations at the low level of the pelagic food chain.

Since in the pelagic systems the photosynthetic picoplankton, such as cyanobacteria and small eukaryotic phytoplankton, heterotrophic bacteria and protozoan consumers overlap in size, it is difficult to separate their different functional categories for experimental purposes. However, it has been demonstrated that heterotrophic nanoplankton, such as nanoflagellates and ciliates, are the primary consumers of the bacterioplankton in the open sea (Fenchel, 1982; Estep *et al.*, 1986), and therefore are regulating bacterioplankton population abundance (Azam *et al.*, 1983).

Some studies on bacterioplankton growth and grazing by protozooplankton have been made, but most of them were conducted for heterotrophic bacteria. In this aspect, several methods have been used to estimate the production or grazing mortality of marine bacterioplankton. These include labelling of bacterioplankton with [^{3}H] -thymidine (Fuhrman and Azam, 1980; Hollibaugh *et al.*, 1980; Gast 1985), cell size fractionation (Wright and Coffin, 1984), a combination of the two approaches (Servais *et al.*, 1985), serial dilution (Landry and Hassett, 1982; Ducklow and Hill, 1985), ingestion of fluorescent latex beads (Bird and Kalff, 1986; Borsheim, 1984), frequency of dividing-divided cell (Davis and Sieburth, 1984) and selective metabolic inhibitors (Fuhrman and McManus, 1984; Newell *et al.*, 1983).

Information about cyanobacteria growth rates and the grazing pressure by heterotrophic nanoplankton are relatively limited, but some studies on this aspect have been conducted. The growth rates for *Synechococcus* have been estimated from the increases in chlorophyll concentrations (Bienfang and Takahashi, 1983), from $^{14}CO_2$ incorporation (Douglas, 1984; Iturriaga and Mitchell, 1986) and by direct cell counts in diffusion chambers (Landry *et al.*, 1984). The net specific growth rate of *Synechococcus* in culture has been estimated as up to 1.5/d (Morris and Glover, 1981). Protozoa including the aplastidic flagellates and aloricate ciliates which are smaller than 20 μm in diameter, were found to be the major grazers of *Synechococcus* spp. (Johnson *et al.*, 1982; Sherr *et al.*, 1986). Furthermore, the specific grazing rates ranged from 0.1 to 0.4/d have been determined for a mesotrophic system (Landry *et al.*, 1984).

Four major approaches have been recently derived from heterotrophic bacteria studies to measure cyanobacteria growth rate and grazing mortality in marine environments: 1) sample serial dilution to reduce grazing pressure (Landry *et al.*, 1984; Campbell and Carpenter, 1986a); 2) growth rate calculation based on the frequency of dividing cell (FDC) of *Synechococcus* (Campbell and Carpenter; 1986b); 3) growth rates and grazing losses determined by the means of cellular carbon incorporation and ^{14}C-labeled sample addition (Iturriaga and Mitchell, 1986) and 4) inhibiting cyanobacteria growth by selective metabolic inhibitors and allowing direct measurement of grazing (Campbell and Carpenter, 1986a).

In the first technique, it is assumed that predator grazing rates are not affected by cyanobacteria

densities. When the sample is diluted with cell-free water to reduce the grazer density, the net growth rate should increase proportionally and grazing rate can therefore be calculated. The second technique (FDC) is based on the relation between number of cells in a given stage of division cycle and growth rate and upon the assumption that the duration of division is constant with respect to changing environmental conditions. The third technique for growth rate measurements is based on a relative increase of cellular carbon concentration (ICC) during a experiment. For grazing loss measurements the experiments were conducted by adding ^{14}C-labelled culture cyanobacteria cell (LCC) to seawater sample and incubating *in situ* and in the dark. According to total ^{14}C -*Synechococcus* label added and grazer label after incubation the grazing loss rate can be calculated. In the last technique, ampicillin (an inhibitor of prokaryotic cell division) is used to hold *Synechococcus* population at a constant level. This inhibitor does not affect eukaryotic grazers. During the experimental period evolution of the cell number is monitored. As a control, both ampicillin and cycloheximide (a specific inhibitor of protein synthesis in eukaryotes, which has been verified not to affect prokaryotes, Newell *et al.*, 1983; Sherr *et al.*, 1986) are used to confirm that the cyanobacteria number remains constant in the absence of grazing by heterotrophic nanoplankton. This method has been used by Campbell and Carpenter (1986a) to estimate the specific grazing rates on *Synechococcus* spp. in the Long Island Sound and the Northwest Atlantic Ocean.

Although above approaches are different, the calculations of specific growth and grazing loss rates are derived from a general exponential relation:

$$\ln\ (N_t / N_0) = rt, \tag{1}$$

where N_0 = cell abundance at time zero; N_t = cell abundance at time t and r = specific rate. When both growth and grazing occur simultanuously, r, the apparent rate of increase, is a balance between growth, k, and mortality due to grazing, g. Therefore:

$$r = k - g = \ln\ (N_t / N_0)\ / t. \tag{2}$$

However, the exact calculation method for both specific growth rate and grazing rate varies somewhat from one approach to the other. In the dilution method it is assumed that the growth rates (k) of cyanobacteria population are unaffected by cyanobacteria poulation density or by removal of predators, while the mortality rate is proportional to predator density. Thus, in serial diluted samples, the measured rate of population (*Synechococcus*) change (r') is:

$$r' = k - xg, \tag{3}$$

where the factor x = ratio of the mean predator densities in the diluted and natural conditions (Landry *et al.*, 1984).

In the case of the FDC technique, since *Synechococcus* populations are found to divide periodically during the diel cycle (Waterbury, 1984), the equation of McDuff and Chisholm (1982), which is derived for a population grown under a light: dark cycle, has been used to calculate the absolute growth rate:

$$k = \frac{1}{nt_d}\sum_{(i=1)}^{i=n}\ln(1 + f_i), \tag{4}$$

where k = specific growth rate (d^{-1}); f_i = fraction of dividing cells for a given sampling interval; n

= number of sampling intervals and t_d = duration of cell division stage.

When the third technique (ICC) is used for determing the cyanobacteria growth rates, k (d^{-1}) can be calculated by the following equation (Iturriaga *et al.*, 1986):

$$k = \ln [(C\ cell^{-1} + \triangle C cell^{-1} d^{-1})/C\ cell^{-1}], \quad (5)$$

where C symbolizes carbon. The cellular carbon concentration is assumed as 0.121pgC μm^{-3} (Douglas 1984), and a median diameter of 1.0 μm is used for the calculation of *Synechococcus* cell volumes. For grazing experiments using LCC technique, the following equation has been used for the calculation of grazing losses, g (d^{-1}):

$$g = \ln \left| \frac{1}{1 - \frac{CPM_G}{CPM_T}} \right|, \quad (6)$$

where CPM_T = total ^{14}C- *Synechococcus* label added; CPM_G = grazer label retained onto a 8.0μm filter after a 24 h incubation in the dark at *in situ* temperature.

Among these methods the selective metabolic inhibitor method appears to produce the most reliable results, as has been demonstrated by Campbell and Carpenter (1986a). No studies, however, have been done on simultaneous measurements of *Synechococcus* growth rates and grazing disappearance rates using selective inhibitor technique.

The goal of the present study is to improve the selective metabolic inhibitor method to allow simultaneous estimates of *Synechococcus* growth rates and grazing pressure and their diel pattern as well as evolution of the cell properties, such as cell size and phycoerythrin content, during the periods of observations, in order to understand the cycling of *Synechococcus* biomass in the English Channel and the Celtic Sea.

MATERIAL AND METHODS

Sampling and sample treatments

Field experiments. Growth and grazing experiments were conducted in March, August, September and October 1987 at the Estacade Station of Roscoff (48°43′N, 3°59′W) on the south coast of the English Channel and in May 1987 during the Rosimer cruise at Station 421 (49°00′N, 10°03′W) in the Great Sole Bank of the Celtic Sea of the Northeast Atlantic Ocean (Fig. 1).

Surface seawater samples were collected with Niskin bottles (General Oceanics). Each sample was given 4 different treatments: no alteration (except for Rosimer experiment), cycloheximide (100 mg/dm^3, Sigma Chemical Co.) addition alone, ampicillin (Sigma Chemical Co.) addition alone (5 mg/dm^3 in March and May and 0.1 to 5 mg/dm^3 in remaining months) and addition of both ampicillin and cycloheximide as a control. In order to observe the grazing rates of pico-eukaryotic grazers on cyanobacteria, in some experiments water samples were prefractionated into two subsamples through a Nuclepore filter with a pore size of 2 μm at a low vacuum (less than 150 mm Hg) to remove the cells larger than 2 μm. All the treatments were performed on duplicate samples.

During the Rosimer cruise, 60 cm^3 samples were incubated in glass bottles in a deck incubator

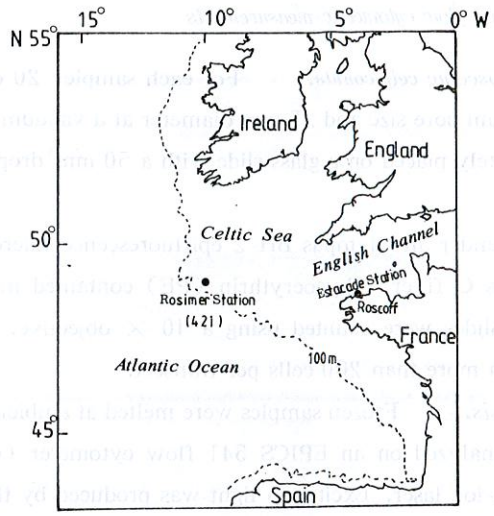

Fig. 1. Chart showing position of the two experimental stations: Estacade Station of Roscoff in the south coast of English Channel and Rosimer Station (421) in the Celtic Sea of the Northeast Atlantic Ocean. The dotted line shows 100 meters contour.

cooled with running surface seawater under sun light. At the Estacade Station of Roscoff, 200 cm^3 samples were incubated *in situ*. In order to compare the diel patterns of growth rates and grazing pressure of *Synechococcus* spp., some incubations began in the morning and some at sunset. All incubations were continued for at least 24 h and in a few cases for up to 96 h.

At 6 or 12 h intervals, duplicate 1 cm^3 subsamples were taken for flow cytometric cell counts during the incubations at Rosimer Station. At the same intervals, duplicate 20 cm^3 subsamples were collected for epifluorescence microscopic cell counts during the experiments at Estacade Station. Besides, for cell size and pigment (phycoerythrin-PE) content measurements using flow cytometry during October experiments, 10 cm^3 subsamples were taken and centrifuged at 10 000 rpm for 6 min. The supernatant was carefully removed and 0.5 cm^3 of concentrated samples to be used. All the subsamples were preserved with 25% glutaraldehyde (MERCK-Schuchardt) at a final concentration of 1% and quickly frozen in liquid nitrogen until analysis.

Laboratory monitoring experiments. A pure *Synechococcus* spp. culture Roscoff 03 and a pure photosynthetic eukaryote culture Roscoff 09 were used for the control experiments. These cultures were maintained in batches in f/2 medium (Guillard & Ryther 1962) at 20℃ under a continuous illumination with a light intensity of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. In both cultures the growth was monitored in the additions of ampicillin (5 mg/dm^3) or cycloheximide (100 mg/dm^3).

Calculations. The formula (2) (see above) was used to calculate absolute growth rate (k) and grazing mortality rate (g) of *Synechococcus* spp. k was calculated from the rate of the increased cell concentration in the cycloheximide treatment, while g was calculated from the disappearance rate of cell in the ampicillin treatment minus the control.

Epifluorescence microscopic and flow cytometric measurements

Epifluorescence microscopic cell counts. For each sample, 20 cm^3 were filtered onto a black Nuclepore filter with 0.2 μm pore size and 25 mm diameter at a vacuum below 200 mm Hg. The Nuclepore filter was immediately placed on a glass slide with a 50 mm^3 drop of sample water and covered with a coverslip.

Slides were counted under an Olympus BH-2 epifluorescence microscope. Green excitation was obtained with an Olympus G filter. Phycoerythrin (PE) contained in *Synechococcus* cell fluoresced brightly orange-yellow. Slides were counted using a 40 × objective. Usually three transects were counted on each filter with more than 200 cells per transect.

Flow cytometric analysis. Frozen samples were melted at ambient temperature and maintained in ice bath. They were analyzed on an EPICS 541 flow cytometer (Coulter, Hialeah Florida) equipped with a 6 W argon-ion laser. Excitation light was produced by the 488 nm line at a power of 1.3 W, focused through a confocal lens, which generated a higher light output than standard lens. Forward angle light scatter (FALS) which reflects particle size (Hutter and Eipel, 1978) was collected by the photodiode. Light scatter at 90° angle (LS90) was collected after reflection on a 488 nm dichroic filter. This signal is dependent on both cell size and cell optical properties (Kerker, 1983, but it appears to be proportional to cell valume (Vaulot, unpublished). Orange fluorescence was measured between 530 and 590 nm and it is proportional to phycoerythrin (PE) content (Glibert *et al.*, 1986). The three signals were amplified on a three - decades logarithmic scales.

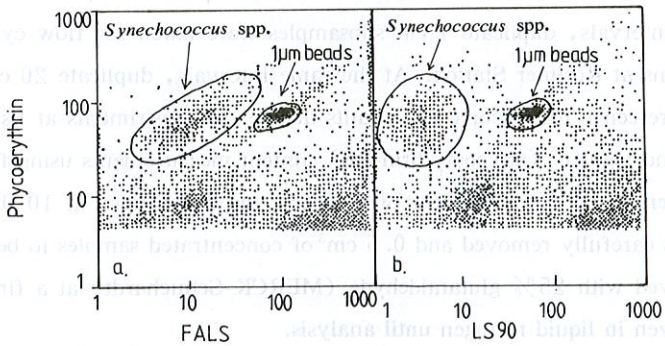

Fig. 2. Distribution of *Synechococcus* cell size, as measured by forward angle light scatter (FALS) and light scatter at 90° angle (LS90), and phycoerythrin (PE) fluorescence obtained by flow cytometry for a 0.4 cm^3 sample collected at Estacade Station, in October 24, 1987. In the two parameter histograms of both FALS - PE fluorescence a) and LS90 -PE fluorescence b), the axes are graduated in relative units on a 3-decade logarithmic scale. Contoured region in the left side corresponds to *Synechococcus* cell population. The population in the upper-right quadrant corresponds to 1μm fluorescent beads added to the sample as internal standard.

Duplicate of 0.2 to 0.5 cm^3 for each sample, to which uniform green fluorescent beads (diameter

1 μm, polysciences, Warring to PA) were added as internal standards, were analyzed. Two-parameters distributions of LS90 vs. PE-fluorescence and FALS vs. PE-fluorescence were recorded and analyzed either on the EPICS MDADS System or on a MS-DOC computer using the CYTOPC Analysis Software (SBR, Roscoff). *Synechococcus* cells appeared in the left part of the two-parameters histogram, as confirmed by analysis of pure *Synechococcus* cultures, while the 1 μm beads appeared in the upper right position (Fig. 2). On each distribution of the average value of the FALS, LS90 and PE-fluorescences, the *Synechococcus* population was computed and rapported to those of the standard fluorescent beads.

RESULTS

Methodological experiments

Laboratory culture controls. Preliminary control experiments with *Synechococcus* strain Roscoff 03 demonstrated that ampicillin (5 mg/dm^3) prevented cell division in this prokaryote without cell lysis, while cycloheximide (100 mg/dm^3) had no significant effect on *Synechococcus* growth (Fig. 3; $p > 0.05$, F-test). On the contrary, control experiments with the eukaryote Roscoff 09 indicated that cycloheximide (100 mg/dm^3) prevented the cell growth without leading to cell lysis , while ampicillin (5 mg/dm^3) had no significant effect on the growth of this eukaryote (Fig. 4; $p > 0.05$, F-test) .

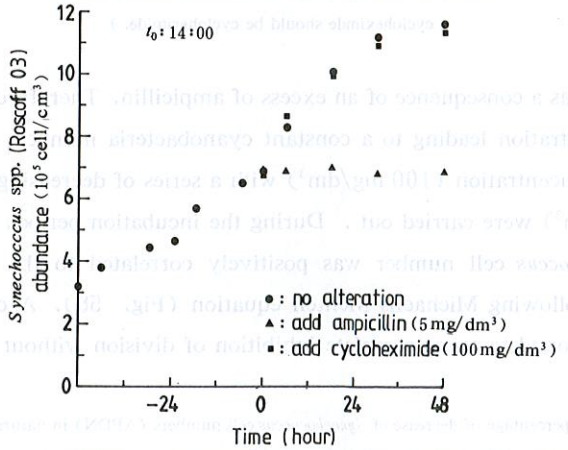

Fig. 3. *Synechococcus* strain Roscoff 03. Control experiments to determine effect of ampicillin (5 mg/dm^3) on cell division and that of cycloheximide (100 mg/dm^3) on cell growth.
(Note: *Synechocccus* should be *Synechococcus* in the figure.)

Optimal ampicillin concentrations. During the experiments in Roscoff, it was found when both cycloheximide (100 mg/dm^3) and ampicillin (5 mg/dm^3) were added to natural seawater samples, *Synechococcus* cell concentrations would decrease during the 24 h incubation period in opposition to the expected behavior, although 5 mg/dm^3 of ampicillin is the usual concentration for the control treatment of *Synechococcus* (Campbell and Carpenter 1986a). This was probably attributed to

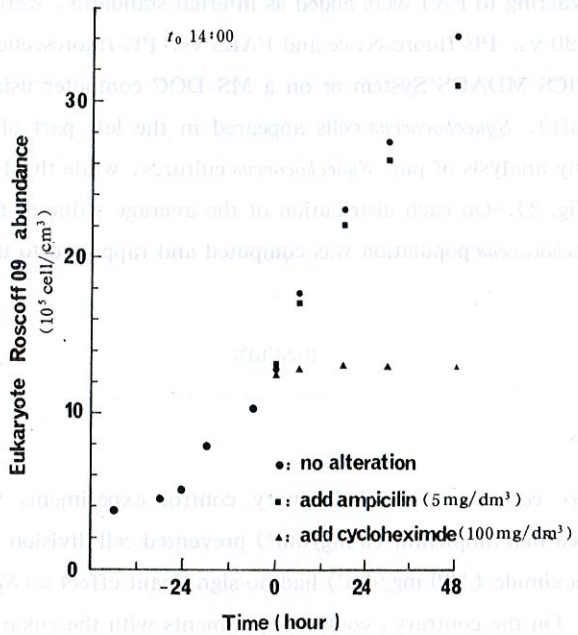

Fig. 4. A eukaryote strain Roscoff 09. Control experiments to determine effect of cycloheximide (100 mg/dm^3) and ampicillin (5 mg/dm^3) on cell growth.
(Note: In the legend ampicilin should be ampicillin and cycloheximde should be cycloherimide.)

Synechococcus cell lysis as a consequence of an excess of ampicillin. Therefore, in order to select an optimal ampicillin concentration leading to a constant cyanobacteria number, experiments combining a fixed cycloheximide concentration (100 mg/dm^3) with a series of decreasing ampicillin concentrations (from 5 to 0.1 mg/dm^3) were carried out. During the incubation periods the average percentage of the decreased *Synechococcus* cell number was positively correlated to the ampicillin concentrations (Table 1, Fig. 5a) following Michaelis Menten equation (Fig. 5b). A concentration of 0.1 mg/dm^3 of ampicillin was found to cause complete inhibition of division without any cell lysis.

Table 1. Average percentage of decrease of *Synechococcus* cell numbers (APDN) in natural samples in presence of cycloheximide (100 mg/dm^3) and different concentrations of ampicillin (n, experimental numbers)

Ampicillin (mg/dm^3)	APDN (% d^{-1}) (mean ± SD)	n
5.0	51±20	5
2.5	50±26	3
1.0	47±25	4
0.5	31	1
0.25	24±4	2
0.1	4±4	4

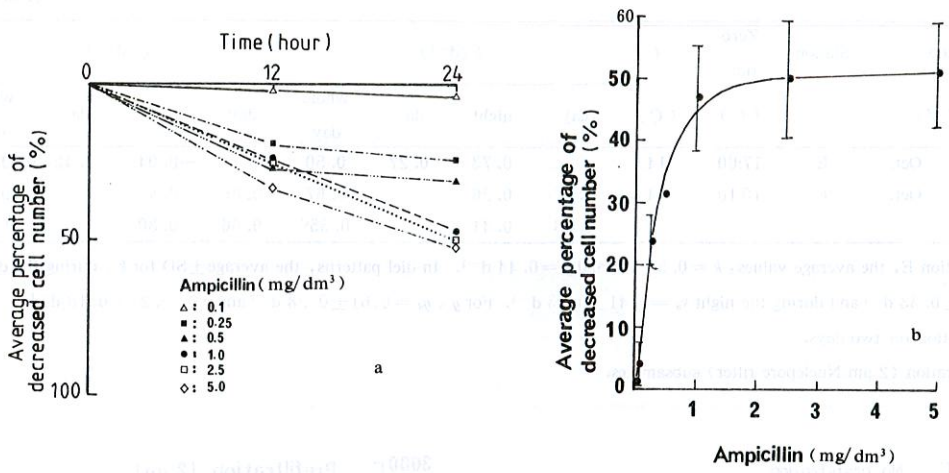

Fig. 5. Average percentage of decreased *Synechococcus* cell number (APDN) under the addition of cycloheximide (100 mg/dm³) with different concentrations of ampicillin (0.1 to 5 mg/dm³). a) Diel disappearance of *Synechococcus* and b) APDN vs. ampicillin concentrations added with unalterated cycloheximide concentration (100 mg/dm³).

Sample prefiltration. In order to compare the effects of prefiltration on growth rates and grazing disappearance rates of *Synechococcus*, two experiments were done using the samples taken at Estacade Station. The results obtained show that the growth rates in the prefiltration subsamples were quite close to that in the no prefiltration subsamples (0.73 vs. 0.72 d^{-1} and 0.55 vs. 0.67 d^{-1} respectively), but the grazing rates were lower in the former than in the latter (0.27 vs. 0.64 d^{-1} and 0.46 vs. 0.63 d^{-1}, Table 2). This difference was also found from *Synechococcus* abundance evolutions for ampicillin treatment that showed a larger dropping slope in no prefiltration subsamples than in prefiltration ones (Fig. 6a, b).

Table 2. Daily instantaneous growth (k) and grazing mortality (g) rates for phycoerythrin-containing *Synechococcus* population at the Estacade Station (E)[a] in Roscoff (France) and Rosimer Station (R) in the Northeast Atlantic Ocean

Date (1987)		Station	Zero time (t_0)	t (°C)	k (d^{-1}) day	night	day	whole day	g (d^{-1}) day	night	day	whole day
27	Mar.	E	9:00	9	0.42	0.44		0.43	0.83	0.10		0.46
30	Mar.[b]	E	19:00	9		0.19	0.31	0.25		0.02	1.08	0.55
				9		0.18	0.42	0.30		0.10	0.31	0.21
7	May	R	16:00	12		0.59	0.05	0.32		0.34	0.48	0.41
1	Aug.	E	10:00	17	1.12	0.32		0.72	0.92	0.41		0.64
								0.73[c]				0.27[c]
7	Aug.	E	18:00	16		0.36	0.72	0.54				
17	Sep.	E	18:30	17		0.79	0.52	0.65				
23	Sep.	E	10:00	16	0.33	0.19		0.26				

(*To be continued on the next page*)

(*Continued*)

Date (1987)		Station	Zero time (t_0)	t (°C)	k (d^{-1})				g (d^{-1})			
					day	night	day	whole day	day	night	day	whole day
24	Oct.	E	17:00	14		0.73	0.27	0.50		−0.04	0.42	0.19
28	Oct.	E	10:10	14	1.07	0.26		0.67	0.85	0.42		0.63
					0.68	0.41		0.55[c]	0.66	0.30		0.46[c]

a. At Station E, the average values; $k = 0.51\ d^{-1}$ and $g = 0.44\ d^{-1}$. In diel patterns, the average±SD for k, during the day $k_d = 0.54 \pm 0.33\ d^{-1}$ and during the night $k_n = 0.41 \pm 0.23\ d^{-1}$. For g, $g_d = 0,61 \pm 0.28\ d^{-1}$ and $g_n = 0.21 \pm 0.18\ d^{-1}$.

b. Incubation for two days.

c. Prefiltration (2 μm Nuclepore filter) subsamples.

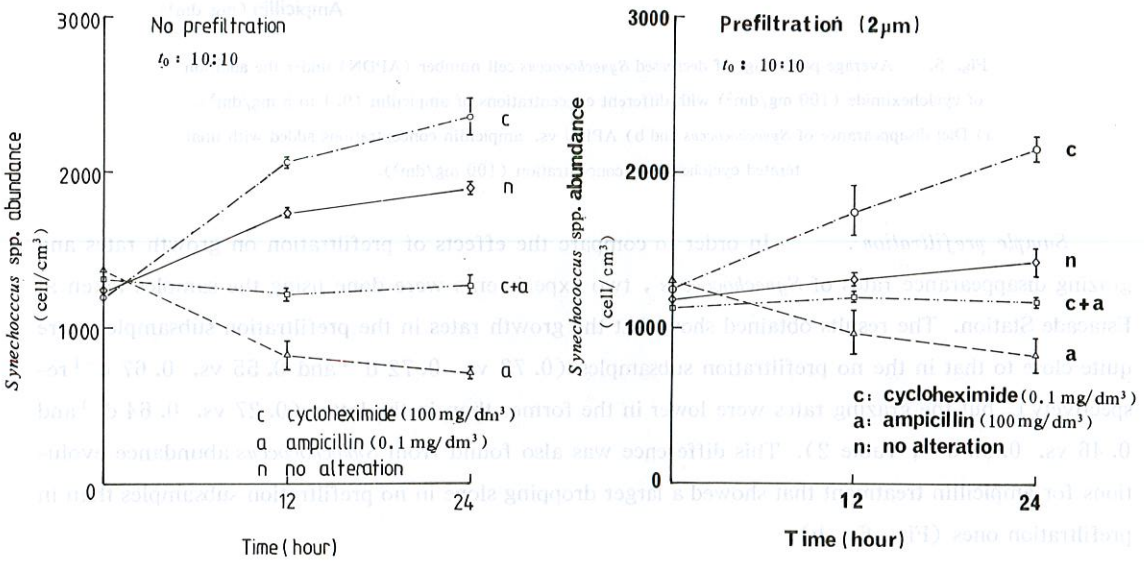

Fig. 6. The evolutions of *Synechococcus* cell concentration after the four different treatments.
a) no prefiltration group and b) prefiltration (2 μm pore size Nuclepore filter) group.

Field experiments

Growth rates and grazing mortality rates. Specific growth rates (k) and grazing rates (g) measured in different times during the year and at both Estacade and Rosimer Stations were summarized in Table 2. The growth rates ranged from 0.25 to 0.72 d^{-1} (not including prefiltration samples and culture). The average values at Estacade Station were 0.51 d^{-1} for growth rate and 0.44 d^{-1} for grazing mortality rate with a range of the latter from 0.21 to 0.64 d^{-1}. At Rosimer Station the values of both k (0.32 d^{-1}) and g (0.41 d^{-1}) were lower than the averages at Estacade Station. The growth and grazing evolutions during the experiments at the two stations were shown in Fig. 7. In growth

treatment cycloheximide inhibited eukaryote grazer (heterotrophic nanoplankton) to allow *Synechococcus* cell growth was measured directly. On the other hand, in grazed treatment ampicillin prevented division of *Synechococcus* cell, thus, the cell disappearance rate (owing to grazing) was measured directly.

Diel patterns of Synechococcus growth rates and grazing mortality rates. An obvious diel pattern of grazing mortality rates was observed. In all the eight experiments for measurements of the grazing disappearance rates, the rates during the day (the mean ± SD : $0.61 \pm 0.28\ d^{-1}$) were higher than those during the night (the mean ±SD : $0.21 \pm 0.18\ d^{-1}$). The difference was significant ($p < 0.01$) by the t-test (Table 2). For growth rates the results were more mixed; among eleven experiments there were 7 experiments in which the growth rates were higher during the day than during the night. The average value of growth rates during the day ($0.54 \pm 0.33\ d^{-1}$) was also higher than that during the night ($0.41 \pm 0.22\ d^{-1}$), but the rate difference between day and night was not significant ($p < 0.3$). The diel patterns of *Synechococcus* growth rates and grazing mortality rates could also be observed from the slopes of the cell abundance evolutions (Figs 6, 7).

Variations of cellular characteristics of Synechococcus during the growth and grazing experiments. The average size (measured by forward angle light scatter-FALS and light scatter at 90° angle-LS90) and PE content (measured by orange fluorescence) of *Synechococcus* cells were determined with flow cytometry.

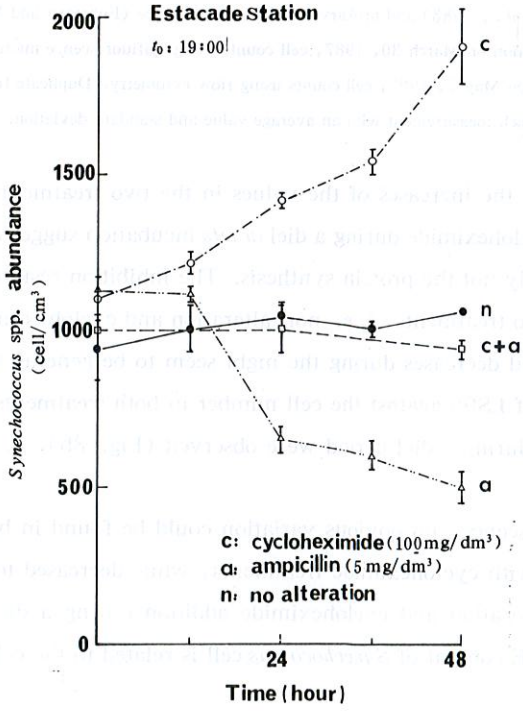

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Fig. 7a

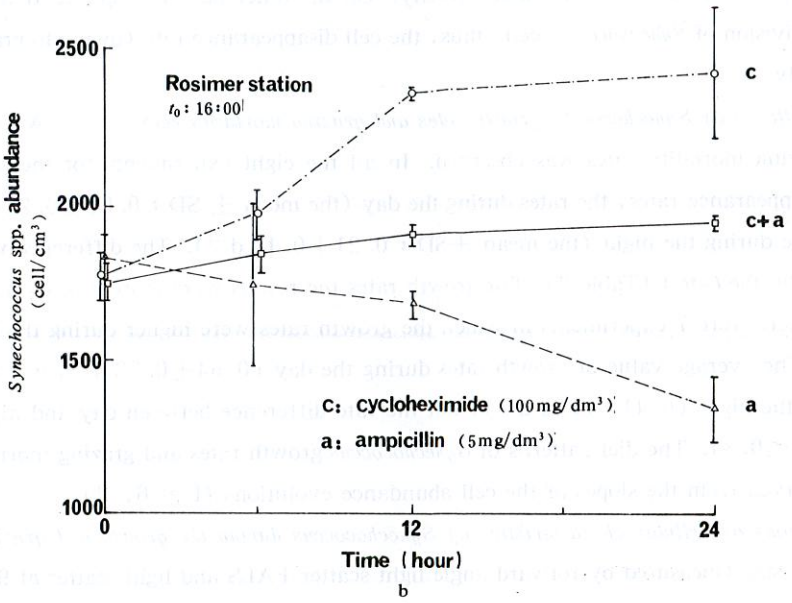

Fig. 7. Daily simultaneous growth and grazing experiments using the selective eukaryote-inhibitor technique (Newell *et al*., 1983) and prokaryote-inhibitor technique (Fuhrman and McManus, 1984). a) at Estacade Station on March 30, 1987, cell counts using epifluorescence microscopy and b) at Rosimer Station, on May 7, 1987, cell counts using flow cytometry. Duplicate treatment was for each measurement with an average value and standard deviation.

For FALS parameter, the increases of the values in the two treatments of addition of ampicillin and both ampicillin and cycloheximide during a diel *in situ* incubation suggested that ampicillin inhibited the cell division but probably not the protein synthesis. The inhibition resulted in an increased cell size (Fig. 8a). In the other two treatments, i. e. non-alteration and cycloheximide addition; the increases of FALS during the day and decreases during the night seem to be general (Chisholm *et al*. 1986).

Reverse fluctuations of LS90 against the cell number in both treatments of non-alteration and the addition of cycloheximide during a diel period were observed (Fig. 8b). It infers a decreased cell size from increased growth rate.

For the orange fluorescence, no obvious variation could be found in both additions of ampicillin and combining ampicillin with cycloheximide treatments, while decreased tendencies were observed in both treatments of non-alteration and cycloheximide addition during a diel incubation period (Fig. 8c). It suggests that the PE content of *Synechococcus* cell is related to the cell volume, therefore to the growth rate.

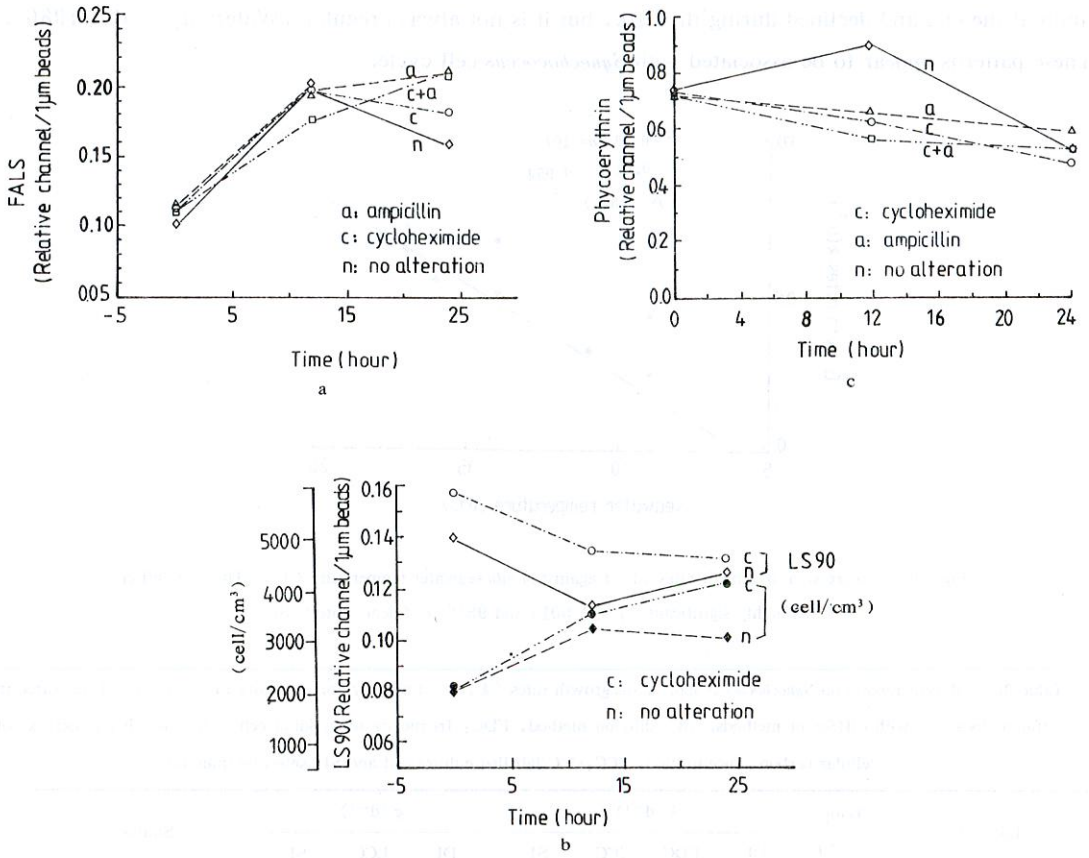

Fig. 8. Evolution of cellular characteristics determined with flow cytometry. a) Forward angle light scatter (FALS), b) light scatter at 90° angle (LS90) and c) PE-fluorescence. The symbols, n: no alteration, a: addition of ampicillin, c: addition of cycloheximide and a+c: addition of ampicillin with cycloheximide.

DISCUSSIONS

The specific growth rates observed in this study (Table 2) can be compared with others (Table 3). The variations are likely attributed to the different methodology used by different authors and also to various environmental conditions, such as temperature, light availability, nutrients, under which the experiments were done. A positive close correlation ($r = 0.88$) between diel growth rates and *in situ* seawater temperature which ranged from 9 to 20℃ were observed in the present experiments (Fig. 9) and the correlation was highly significant ($p < 0.001$). This suggests that higher temperature results a higher physiological activity of cyanobacteria. Such relation has also been observed for natural phytoplankton assemblages in the Bedford Basin (Harrison and Platt, 1980) and in the Chesapeake Bay (Harding *et al*., 1986), but no publications could be found specially for *Synechococcus* spp.

In relation to the diel patterns, our results show that in most of the cases the growth rates were higher during the day than the night. This was coincident with those reported by Chisholm (1981)

who found that *Synechococcus* cell division rate often increased during the light period, reached a maximum at the end and declined during the dark, but it is not always regular (Waterbury *et al.*, 1986). These patterns appear to be associated with *Synechococcus* cell cycle.

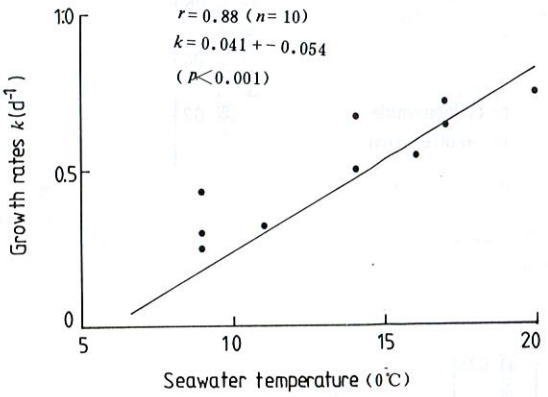

Fig. 9. Regression of growth rates (d^{-1}) against *in situ* seawater temperature (℃). The correlation was highly significant ($p < 0.001$) and 95% confidence intervals.

Table 3. Recent reports on *Synechococcus* spp. *in situ* growth rates (k, d^{-1}) and grazing mortality rates (g, d^{-1}) measured in different locations with diffferent methods. DL: dilution method, FDC: frequency of dividing cell technique, ICC: increase of cellular carbon concentration, LCC: ^{14}C-labelled culture cell and SI: selective inhibitor.

Location	Temp. (℃)	k (d^{-1})				g (d^{-1})			Source
		DL	FDC	ICC	SI	DL	LCC	SI	
Coast of Nor. Atlantic	25	0.84	0.57			0.83		0.70	Campbell and Carpenter (1986a)
Long Island sound	25	0.78				0.22			d°
Kaneohe Bay, Hawaii		1.70				0.26			Landry *et al.* (1984)
Nor. Central Pacific Gyre				1.63			0.30		Iturriaga and Mitchell (1986)
Sargasso Sea	22		0.73						Campbell and Carpenter (1986b)
Gulf of Maine	15		0.53						d°
Wilkinson's Basin	20		0.45						d°
Caribbean Sea	26		0.56						d°
Coast of Eng. Channel	14				0.51			0.44	this study
Northeast Atlantic	12				0.32			0.41	d°

The specific grazing mortality rates observed in this study (Table 2) can be also compared with others (Table 3). It seems that the average grazing rate ($0.44d^{-1}$) being close to the average growth rate ($0.51d^{-1}$) suggests a rapid recycling of *Synechococcus* biomass. In the diel patterns, our results exhibit that the grazing mortality rates were higher during the day (mean$=0.61d^{-1}$) than during the night (mean$=0.21d^{-1}$) in all the experiments. It may be attributed to the attraction of dissolve organic matter excreted by *Synechococcus* during the photosynthesis in the day for heterotrophic nanoplankton (Sieburth, 1982) or to some clock-induced rhythmicity of grazer behaviour. Wikner *et al*. (1990) reported that high grazing rates of flagellates on bacteria were often encountered at around midday and the low rates in the early morning and at midnight. They found that the periods with the low rates were corresponding to sharp increases in flagellate abundance, suggesting that the flagellates ceased feeding during their cell division. In contrast with the effects of diel alteration on the grazing pressure, the diel grazing rates were relatively independent of temperature between 9 and 17℃ in this study. This phenomenon has also been observed in the experiments estimating the effects of temperature on the feeding rate of coastal ciliated protozoa made by Stoecker and Guillard (1982), who found that dinoflagellate clearance rates by a coastal tintinnid were relatively independent of temperature between 8 and 20℃ with a Q_{10} response of 1.0 to 1.1.

Size fractionation yielded a significant result. The grazing rates measured in the seawater samples, which had been prefiltered through a 2 μm Nuclepore filter, were still present but were reduced (Table 1, Fig. 6b). This indicates that the heterotrophic picoflagellates smaller than 2 μm were responsible for the grazing. Furthermore, the faster growth observed in 2 μm prefiltered samples treated with cycloheximide compared to that in not treated sample, may also be attributed to the eukaryotic grazers in the filtrates (Fig. 6b), as the similar phenomena have been observed for heterotrophic bacteria grazing mortality by Newell *et al.*, (1983) and Fuhrman and McManus, (1984). Estep *et al*. (1986) reported that chloroplast-containing chrysomonads, predominant new species *Ochromonas* and *Chrysamoeba*, with a size 2 μm in diameter preyed on heterotrophic bacteria and cyanobacteria. Moreover, grazing has been reported even in 1 and 0.6μm Nuclepore filtrates. It is probably due to small or flexible flagellates or some other protozoa which can pass through 0.6μm filter. These bacteria-sized eukaryotes have been estimated to be responsible for more than 50% of the total grazing in the coastal waters (Fuhrman and McManus, 1984).

Although several techniques, described earlier, have been used to measure *Synechococcus* growth rates and grazing mortality rates, methodology appreciation and choice of accurate techniques are very important for studying *Synechococcus* biomass cycling, population dynamics and the quantitative relations between *Synechococcus* and its predators in the microbial food web. Dilution technique is based on the assumptions that predator grazing rates are identical after dilutions and in natural conditions, i.e., they are independent of prey densities. In fact, the grazing of the heterotrophic nanoplankton population has been found to be increased proportionately with cyanobacteria concentrations (Landry *et al.*, 1984). Therefore, this method results in underestimates of the grazing rate and overestimates of the growth rate, especially at low cell densities.

The advantage of the FDC technique (frequency of dividing cell technique) is to get rid of incu-

bations. However, this technique relies on a main hypothes, i. e., the duration of the division stage (or called "septa formation time") t_d is constant and independent of changing environmental conditions. But this could not be coincident with all actual states. Waterbury *et al*. (1986) demonstrated that t_d increases with decreasing temperature in cells grown in continuous light. Campbell and Carpenter (1986b) reported that t_d varied from 2 to 4 h was observed to be depended on the clone. Therefore, it must be measured with culture in the lab, but it is difficult to correspond to that *in situ* and even though a one or two hour error in the estimate of t_d can cause large errors in the estimate of k, especially for small values of t_d (Chisholm *et al*., 1986). Besides, the grazing effects may also cause the FDC-based growth rate to be underestimated.

When the growth rates are measured using the third method (ICC), the assumption on cellular carbon concentration being a constant (0.121pgC μm^{-3}) may induce a large error and the growth rates are probably overestimated, due to the neglect of respiration by the standard photosynthetic techniques (Williams *et al*., 1983). For grazing loss measurements using LCC method, the rates are probably underestimated due to small grazer loss and grazer excretion and respiration of metabolized label being neglected (Iturriaga and Mitchell, 1986).

The metabolic inhibitor method provides in general good results. However, the specificity of inhibitors is critical. The efficiency of cycloheximide which affects synthesis of RNA or protein, can depend on diurnal cell cycles of protozoan (Suhama and Hanson, 1971). This may explain why the growth rates were not always higher during the day than the night. On the other hand, antibiotics such as ampicillin can cause lysis of prokaryotes (Gale *et al*., 1981). This may explain why we observed a decrease in *Synechococcus* abundance at high concentration of ampicillin when cycloheximide was present. A range of inhibitor concentrations has therefore to be carefully tested before the method can be reliably applied.

Despite these problems, the selective metabolic inhibitor technique may produce more reliable results than other techniques. In comparison with others, the selective inhibitor method involves minimal manipulation of water samples, does not require the use of radioisotopes and can also be used for simultaneous estimates of cyanobacteria growth rates and grazing pressure.

CONCLUSIONS

Accurate estimates of growth rates and grazing pressure of cyanobacteria are important for understanding the food chain dynamics. In this study in the coast of European waters, the grazing mortality rate (mean=0.44d^{-1}) was very close to the average growth rate (mean=0.51d^{-1}). It suggests that the biomass is rapidly recycled and *Synechococcus* are an important trophic link in the pelagic food web. Furthermore, the existence of bacteria-size grazer would probably lead to longer food chains, as a result, the classical conceptions on the structure of marine ecosystems may have to be revised.

In order to understand the contribution of *Synechococcus* spp. to the primary production of phytoplankton assemblage and the biomass transformation efficiency of cyanobacteria to heterotrophic nanoplankton in the food chain, it is necessary to combine the estimates of growth and grazing mortali-

ty rates with size-fractionated productivity measurements and quantifications of heterotrophic nanoplankton.

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