Pico- and ultraplankton Sargasso Sea communities: variability and comparative distributions of *Synechococcus* spp. and algae*

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ABSTRACT: Ultraplankton (0.2 to 5 μm) provided >88% of euphotic layer chlorophyll (Chl) at 2 Sargasso Sea stations in July/August 1986. Communities were further characterized to quantify the separate abundances of phycoerythrin-fluorescing cyanobacteria *Synechococcus* spp. and Chl-fluorescing algae in 0.2 to 0.6, 0.6 to 1 and 1 to 5 μm size fractions. Throughout the water-column at both stations, the majority of *Synechococcus* cells were consistently found in the 0.6 to 1 μm fraction; the Sargasso Sea WH7803 serogroup was not a dominant component of *Synechococcus* populations at any depth. Highest numbers of *Synechococcus* cells were always in the surface isothermal layer, where they accounted for >95% of all ultraphytoplankton cells. At the base of the euphotic layer total numbers of photoautotrophs were low, but numbers of algae increased. The varying distribution of the 2 ultraphytoplankton components with depth was also reflected in their separate contributions to Chl concentrations, and algae were the main contributors to Chl maxima at 1 to 3% I_0 . Above this depth, a pigment maximum in phycoerythrin (PE) l^{-1} occurred at the nitracline, coincident with a peak in primary productivity. PE maxima were due to an increase in PE content of *Synechococcus* cells and not to an increase in their abundance. The 2 stations did however exhibit significant differences. High surface productivity at Stn 1 was supported by nanomolar changes in nitrate concentrations, which selectively and rapidly induced a *Synechococcus* bloom. In contrast, the water-column characteristics of Stn 2 were relatively stable, *Synechococcus* spp. were less abundant throughout the water-column, while algae were twice as abundant at the Chl maximum and 0.5% light level, where most were of picoplankton size (79 to 89% of algae were in <1 μm fractions). Data suggest that there is a considerable degree of variability in the abundance, composition, and productivity of stratified oceanic ultraphytoplankton communities.

INTRODUCTION

During the last decade, increasing efforts in biological oceanography have been focused on defining and quantifying the microbial components of oceanic rate processes, particularly as they affect the cycling of carbon and nitrogen. Renewed interest in oceanic phytoplankton and primary productivity estimates has been largely stimulated by new information on the predominance and activity of ultra- (<3 μm) and picophytoplankters (<1 μm). The ecological success of

these in oceanic regions has been partly attributed to the increased efficiency of light-harvesting and nutrient uptake conferred by their small cell size (cf. Fogg 1986, Kirk 1986, Raven 1986) and their negligible sinking rates (Takahashi & Bienfang 1983). Revised higher estimates of oceanic primary productivity (e.g. Laws et al. 1987, Marra & Heinemann 1987) have a direct impact upon developing models of biogeochemical cycling of carbon and nitrogen in the upper ocean, where dynamics are driven by synergistic interactions between ultraplankton-size photoautotrophs, chemototrophs, methanotrophs, heterotrophic bacteria and their protozooplankton grazers (cf. reviews by Fogg 1986, Platt & Li 1986, Stockner & Antia 1986).

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More recently, it has become apparent that ultraphytoplankton contains both cyanobacteria and algae. These 2 photosynthetic components can be distinguished in mixed communities and enumerated by direct count epifluorescence microscopy, since red-pigmented cyanobacterial cells have a dominant phycoerythrin (PE) fluorescence emission, while all algal species have a predominant Chl fluorescence emission (Glover 1985). While the contribution of marine cyanobacterial populations of *Synechococcus* spp. to primary production has received considerable attention (see reviews by Glover 1985, Waterbury et al. 1986), direct estimates of both the biomass and photosynthetic activity of similarly-sized algae within the same water mass had never been attempted.

During a cruise to the Sargasso Sea in summer 1986, we used 2 recently developed field techniques to measure nanomolar nitrate concentrations in surface waters and *Synechococcus* PE concentrations throughout the water-column. We also compared the abundance of *Synechococcus* spp. to that of algae in different size-fractions and estimated the separate contributions of these 2 phytoplankton components to the total standing crop of Chl and primary productivity at several depths. Measurements and experiments were also designed to define possible differences in adaptive responses employed by *Synechococcus* spp. and algal ultraplankters ($<5\ \mu\text{m}$) to cope with variations in light and nutrient flux that might explain any distributional differences. This manuscript describes the temporal and spatial variations that we observed in PE and Chl concentrations and the numerical abundance of *Synechococcus* spp. and algae in ultra- ($<5\ \mu\text{m}$) and pico- ($<1\ \mu\text{m}$) phytoplankton communities.

MATERIALS AND METHODS

This study was conducted in the Sargasso Sea (Fig. 1) during the summer of 1986 from the RV 'Endeavor' (Cruise EN-146). Stn 1 was 140 km east of Bermuda ($32^{\circ}42'\text{N}$, $63^{\circ}01'\text{W}$) and was occupied from 20 to 28 July. Stn 2 was 165 km NNW of Bermuda ($33^{\circ}42'\text{N}$, $65^{\circ}22'\text{W}$) and was occupied from 2 to 9 August.

Physical/chemical measurements and sample collection. The research vessel received daily charts of sea surface temperature derived from NOAA satellite images (AVHRR) and the position of the Gulf Stream and cold-core eddies in Fig. 1 were taken from the NOAA/National Weather Service analysis on 18 July. Subsequent thermal maps indicated that these features did not change significantly throughout the cruise.

A submersible pumping system (180 m) was constructed to collect discrete samples, while providing simultaneous continuous vertical profiles of tempera-

Fig. 1. Locations of 2 stations in the Sargasso Sea during RV 'Endeavor' Cruise EN-146, 17 July to 11 August 1986. Position of Gulf Stream (GS) and cold-core eddies (CE) were taken from NOAA/National Weather Service analyses on 18 July

ture, in vivo Chl fluorescence and quantum irradiance (Biospherical design) as a function of depth. One modification over the prior system (Prézelin et al. 1987) was the use of an internal hose diameter of 5 cm, intentionally enlarged to minimize shear effects on phytoplankton populations, and pumping was demonstrated to have no effect on either phytoplankton biomass or photosynthetic activity, when compared to replicate samples collected with GOFLO bottles. The flow rate was ca $113\ \text{l}^{-1}\ \text{min}^{-1}$, with a 3 min transit time from intake to outlet. Pump effluent was directed through an adapted Turner fluorometer to measure in vivo Chl fluorescence. Analog data from the fluorometer, temperature and depth probes, in situ irradiance and daily incident irradiance, (quantum sensor, Biospherical Instruments) were recorded on strip charts.

Samples from discrete depths were collected from the fluorometer outflow into 125 ml polyethylene bottles and frozen for subsequent onshore analysis of nitrate concentrations. Nitrate in the upper 60 m was measured using a chemiluminescent technique (precision of $\pm 2\ \text{nM}$; Garside 1982), and at greater depths by the standard colorimetric method (precision of $\pm 80\ \text{nM}$; Strickland & Parsons 1972). Discrete samples were collected directly from the pump effluent into darkened 20 l polypropylene carboys, that were immediately transported into the shipboard laboratory for analysis. All apparatus used for the collection and incubation of water samples was cleaned by the procedure of Fitzwater et al. (1982). To recycle, the vessels were rinsed with 0.25 N HCl followed by deionized water.

Cell enumeration of photoautotrophs. Numbers of

Chl-fluorescing algae and PE-fluorescing *Synechococcus* cells were determined by epifluorescence microscopy in 3 size-fractions of unpreserved samples separated by differential filtration (Glover et al. 1986). A minimum of 100 cells were counted and standard deviations were 10 to 25 % of the mean count of several microscope fields for 1 to 5 μm and 0.6 to 1 μm assemblages. Standard deviations were however routinely >25 % for 0.2 to 0.6 μm assemblages, principally due to the very low cell counts in this size range. The percentage of total phytoplankton cells represented by *Synechococcus* spp. in each size range is shown in Table 1. Duplicate samples were preserved in buffered formalin and stored in the dark at 4°C for onshore enumeration of 2 *Synechococcus* serogroups by immunofluorescence labelling (Campbell & Carpenter 1987). Antiserum produced against the Sargasso Sea isolate WH7803 was cross-absorbed with cells of the coastal isolate WH8016, and vice versa, before use.

Cell enumeration of bacteria. Numbers of bacteria were also determined in preserved samples, using the acridine orange direct counting method of Hobbie et al. (1977). A minimum of 100 cells were counted and standard deviations were 3 to 21 % of the mean count of several microscope fields.

Phytoplankton pigment concentrations. Duplicate samples were filtered to determine particulate Chl concentrations, the volume (50 ml to 1 l) depending on the in vivo Chl fluorescence signal from the pump profile and the filter pore size. Each sample was divided into 4 portions, which were filtered through separate Nucleopore filters of 5, 1, 0.6 and 0.2 μm pore sizes and filtrates were discarded. Methods for filtration and extraction are described in Glover et al. (1986). Chl concentrations were determined directly for >5 μm communities and

by mathematical subtraction for 1 to 5, 0.6 to 1 and 0.2 to 0.6 μm fractions. Standard errors were routinely <10 % of the mean. Cell-specific Chl concentrations in pure 0.6 to 1 μm *Synechococcus* populations (92 to 100 % of all autofluorescent cells; Table 1), were used in conjunction with cell numbers in the 1 to 5 and 0.2 to 0.6 μm fractions, to estimate *Synechococcus* Chl in these other size ranges. The quantity of algal Chl in 1 to 5 and 0.2 to 0.6 μm fractions could then be calculated by subtracting the amount of *Synechococcus* Chl from the total concentration in each size category. It was only at the Chl max and the 0.5 % light depth that the 0.6 to 1 μm fraction could not be considered a pure population of *Synechococcus*, as they routinely represented <50 % of all the autofluorescing cells in this size range (Table 1). At these depths, we could only estimate cellular Chl concentrations of *Synechococcus* spp. by proportion, using relative numbers of *Synechococcus*:algal cells within the 0.6 to 1 μm fraction. *Synechococcus* Chl may therefore have been overestimated at the base of the euphotic layer, as algae in this size fraction would probably contain more Chl per cell than *Synechococcus* spp. (see Table II of Glover et al. 1987), since the former were probably larger and Chl is one of their primary light-harvesting pigments.

PE concentrations were determined at sea, using the in vivo fluorescence method of Wyman & Carr (1988), which is based on the ability of glycerol to uncouple energy transfer between PE and phycocyanin (Wyman et al. 1985). Samples (1 l) were filtered through Nucleopore filters of 0.6 μm pore size to collect *Synechococcus* cells. Cells were resuspended in 5 ml of 50 % glycerol, excited at 520 nm and PE fluorescence emission was determined at 570 nm in an adapted Turner fluorome-

Table 1. Percentage of autofluorescing phytoplankton cells represented by PE-rich *Synechococcus* spp. within 3 size fractions at various depths in the euphotic zone. Mean value ($\bar{x}$), and range (in brackets) is provided for samples collected from different pump casts over 5 d

Light depth (% I_0)	Station 1			Light depth (% I_0)	Station 2		
	1–5 μm $\bar{x}$	0.6–1 μm $\bar{x}$	0.2–0.6 μm $\bar{x}$		1–5 μm $\bar{x}$	0.6–1 μm $\bar{x}$	0.2–0.6 μm $\bar{x}$
PE _{max}	90	93 (91–96)	100 (99–100)	90	91 (81–95)	99 (98–100)	99 (98–100)
	64	91 (89–94)	99 (99–100)	–	–	–	–
	–	–	–	40	89 (87–92)	99 (99–100)	99 (96–100)
	34	91 (81–97)	99 (98–100)	–	–	–	–
	26	71 (44–86)	98 (98–100)	26	87 (76–94)	98 (97–100)	98 (92–100)
PE _{max}	–	–	–	19	72 (54–87)	98 (96–100)	98 (94–100)
	14	80 (77–85)	98 (97–100)	–	–	–	–
	–	–	–	9	69 (64–78)	95 (91– 98)	91 (81– 97)
	6	77 (70–93)	92 (87– 94)	–	–	–	–
	–	–	–	PE _{max} 4	76 (65–83)	92 (85– 97)	74 (44–100)
Chl _{max}	3	44 (19–68)	62 (34– 81)	–	–	–	–
	0.5	40 (19–78)	66 (42– 77)	Chl _{max} 1.5	31 (23–40)	35 (16– 45)	54 (32–100)
				0.5	25 (13–33)	39 (9– 63)	45 (0– 86)

ter, calibrated with both whole cells and isolated phycobilisomes from the Sargasso Sea *Synechococcus* clone, WH7803. Since the washing procedure did not remove all the cells from the filter, cell numbers were determined in the glycerol suspension and data were corrected accordingly.

Photosynthesis measurements. Diurnal measurements of photosynthesis-irradiance (P-I) relations were conducted on whole communities collected from 8 depths at dawn, midday and dusk. Values reported here are for midday values only. Procedures for filtration, washing and determining rates of ^{14}C fixation are described by Prézélin et al. (1986). Photosynthetic parameters were derived from non-linear curve fits for the P-I data (Prézélin et al. 1986). Independent measurements of P_{max} indicated that one standard deviation was routinely $< 15\%$ of the mean value.

In situ rates of daily primary production were determined for whole communities from 8 depths. ^{14}C -bicarbonate was added to duplicate 230 ml samples in polycarbonate bottles (1.3 to $1.5\ \mu\text{Ci ml}^{-1}$ final concentration). At dawn, bottles were suspended at each depth using an in situ line and float. Bottles were retrieved at dusk (12 h incubation) and 50 ml portions were filtered through Nuclepore filters of $0.2\ \mu\text{m}$ pore size. Additional samples from each depth were also filtered immediately after the addition of ^{14}C -bicarbonate, for zero time controls. One standard deviation ranged from 2 to 16% of the mean value (with one exception of 30%). Procedures for filtration, washing,

and determining photosynthetic rates are provided in Glover et al. (1986).

RESULTS

Both stations were stratified with pigment maxima below the thermocline and more than 88% of the integrated standing crop of Chl was found in ultraplankton (0.2 to $5\ \mu\text{m}$). Vertical profiles of light availability as well as the abundance and composition of ultraphytoplankton assemblages were different at the 2 stations (Figs. 2 to 5). In addition, the water-column characteristics at Stn 2 were relatively stable over the monitoring period, while temporal changes occurred in the surface isothermal layer at Stn 1.

Similarities between stations

Both stations exhibited a number of similar features: An in vivo PE glycerol-uncoupled fluorescence maximum occurred on the 21.5°C isotherm at 3.7 to 6.5% I_0 , in the upper part of the nitracline (Figs. 2 and 3) and coincident with a maximum in midday photosynthetic capacity (Table 2). Below the PE maximum there was an in vivo Chl fluorescence maximum, which followed the 21°C isotherm (Fig. 2) at the base of the nitracline ($>10\ \mu\text{M NO}_3$). Extracted Chl concentrations showed similar depth distributions (Fig. 3), such that the Chl fluorescence:biomass ratio was constant. One excep-

Fig. 2. Depth profiles of temperature and irradiance at the 2 stations. Temperature structure and position of in vivo Chl fluorescence maximum were derived from continuous vertical pump profiles taken at various times (---). Attenuation coefficients (K_d) for each station are mean values obtained from several profiles of downwelling irradiance taken over similar time frames. Position of PE maximum was derived from discrete analyses of samples from the pump outflow. Arrows (1a, 1b and 2) indicate the times of pump casts for collection of data presented in Figs. 3, 5, 6, 7 and 9

Fig. 3. Concentrations of nitrate (left graphs), phycoerythrin (middle graphs) and chlorophyll (right graphs) in water samples from different depths in the euphotic zones of the 2 stations. Temporal changes at Stn 1 are seen by the differences in depth profiles between 20 July (solid symbols) and 25 July (open symbols). Data for Stn 2 are from 2 August

Table 2. Photosynthetic rates and characteristics of whole phytoplankton communities ($>0.2 \mu\text{m}$)

Light depth (% I_0) ^a	Station 1			Light depth (% I_0) ^a	Station 2		
	20 July I_k^b ($\mu\text{E m}^{-2} \text{s}^{-1}$)	20 July P_{max}^c ($\mu\text{g C l}^{-1} \text{h}^{-1}$)	21 July In situ production ^d ($\mu\text{g C l}^{-1} \text{d}^{-1}$)		2 August I_k^b ($\mu\text{E m}^{-2} \text{s}^{-1}$)	2 August P_{max}^c ($\mu\text{g C l}^{-1} \text{h}^{-1}$)	4 August In situ production ^d ($\mu\text{g C l}^{-1} \text{d}^{-1}$)
PE _{max}	90	379	3.20	90	162	0.07	1.63
	64	172	3.77				
	34	103	3.23	40	103	0.05	1.55
	26	138	2.40	26	257	0.14	1.50
	14	42	2.45	19	262	0.25	1.55
PE _{max}	6	76	—	9	282	0.17	1.05
Chl _{max}	5	—	1.87	PE _{max}	4	111	1.26
	3	70	0.74	Chl _{max}	1.5	27	0.28
	0.5	15	0.13		0.5	22	0.38

^a I_0 = surface irradiance
^b $I_k = P_{\text{max}} / (\text{minimum amount of light required to saturate photosynthesis})$
^c Midday rates of light-saturated photosynthesis, from P-I curves
^d Dawn to dusk rates of photosynthesis at in situ irradiances

tion occurred on 2 August at Stn 2, when maximum concentrations of PE and Chl per liter were coincident.

At both stations, *Synechococcus* spp. were most abundant in the surface isothermal layer (Fig. 4), but they represented less than 8% of the total number of prokaryotes, as bacterial abundance was $198 \pm 283 \times 10^6$ cells l^{-1} above the thermocline at both sta-

tions (Fig. 5). Depth distribution studies indicated that high cell numbers of *Synechococcus* occurred in the mid to upper portion of the euphotic zone ($>10\% I_0$) above pigment maxima and the maximum abundance of algal ultraphytoplankters (Fig. 5). Since *Synechococcus* cell numbers were lower at the PE maximum than those in the surface isothermal layer

Fig. 4. Variations in the numerical abundance of *Synechococcus* spp. (black bars) and algae (cross-hatched bars) in ultraplankton assemblages at 4 locations in the water-column over the course of the monitoring periods at Stn 1 (left graph) and Stn 2 (right graph). As pigment maxima were broadly distributed as a function of depth, data presented here from the PE and Chl maxima represent time course changes in phytoplankton communities from the 21.5°C and 21°C isotherms, respectively

Fig. 5. Depth distribution of *Synechococcus* spp. (●), algae (○) and bacteria (+) in ultraplankton assemblages (0.2 to 5 μm) at Stn 1 on 20 July (left graph) and Stn 2 on 2 August (right graph). Depths of the Chl and PE maxima are indicated to the right

(Figs. 4 and 5), the relatively higher PE concentrations at the nitracline maximum (Fig. 3) were due to an increase in cellular PE content. Fig. 6 shows the increase in *Synechococcus* PE cell⁻¹ below the 10 % isolume at both stations. *Synechococcus* spp. accounted for >95 % of all ultraphytoplankton cells in surface waters and they were also numerically dominant at the nitracline PE maximum (Figs. 4 and 5). At the base of the euphotic layer total numbers of photoautotrophs were low, but numbers of algae increased (Fig. 5) and they became a significant component of deepest ultraphytoplankton assemblages (Fig. 4). In contrast, bacterial abundance remained relatively high throughout the euphotic layer (note abscissa scales on Fig. 5).

The distribution of the 2 ultraphytoplankton components with light depth (Fig. 5) was also reflected in their relative contributions to Chl concentrations (Fig. 7). That is, *Synechococcus* spp. were generally the major contributors to the standing crop of Chl in the surface isothermal layer, while ultraplankton algae provided most at the Chl maximum and 0.5 % light level (Fig. 7). At both stations, ultraplankton algae made a significantly greater contribution to integrated water-column Chl than *Synechococcus* spp., while >5 µm algae provided only a minimal part of the total concentration (Fig. 7).

We further characterized the *Synechococcus* components of the ultraplankton by examining their relative abundance in 3 size classes and by using immunofluorescence assays to enumerate 2 different *Synechococcus* serogroups in 0.6 to 1 and 1 to 5 µm fractions. At both stations, the majority of *Synechococcus* cells were consistently found in the 0.6 to 1 µm fraction, while the minority were in the smallest 0.2 to 0.6 µm fraction (Fig. 8). However, the mean size of the population increased with increasing depth (Fig. 8). Immunofluorescent

labelling of populations by antiserum directed against the Sargasso Sea isolate WH7803 showed that there was no apparent difference in the labelling patterns of *Synechococcus* cells in either 0.6 to 1 µm or 1 to 5 µm size-fractions (Fig. 9). Similar results were obtained with antiserum directed against the coastal *Synechococcus* isolate WH8016 at Stn 2 (data not shown). Furthermore, very weak reactions and low percentage labelling at most depths suggests that the oceanic WH7803 serogroup was not a dominant component of populations at either station (Fig. 9) and 75 to 93 % of all *Synechococcus* cells at any depth were not labelled by either antiserum at Stn 2.

Temporal changes at Stn 1

In addition to the PE maximum located at the nitracline, an additional PE maximum was also found within the isothermal layer at 10 m on 20 July at Stn 1 (Fig. 3). It was evident that both PE maxima represented depths of relatively high total primary productivity (Table 2) and cellular photosynthesis (data not shown), with phytoplankton assemblages dominated by *Synechococcus* spp. (89 to 97 % of all <5 µm cells) that were highly enriched in PE (28 and 40 fg PE cell⁻¹ at 64 and 6 % I₀, respectively; Fig. 6). The 10 m PE maximum did not persist. Indeed, the water-column at Stn 1 was in transition throughout our 5 d monitoring period: there was a 1°C warming above the thermocline between 20 and 26 July (Fig. 2). Advective forces did not appear to be involved, since the surface warming could be solely accounted for by local heating (incident solar radiation was 13×10^7 J m⁻² during the period in which 5.3×10^7 J m⁻² of heating occurred). During the same period,

Fig. 6. Pigment concentrations in *Synechococcus* cells as a function of light depth on 20 July at Stn 12 (+---+), and 2 August at Stn 2 (•—•). Left graph represents cellular Chl content in the 0.6 to 1 µm fraction, which could be considered as a pure *Synechococcus* population (>95 % of all phytoplankton cells) except for the bottom 2 depths. Right graph shows PE content of all *Synechococcus* cells >0.6 µm

Fig. 7. Contributions of >5 µm algae, ultraplankton algae and *Synechococcus* spp. to Chl concentrations at specific light depths and to the total integrated water-column Chl at Stn 1 on 20 July and at Stn 2 on 2 August. Concentrations in >5 µm algal assemblages were determined directly from size-fractionated data, while concentrations in ultraplankton algal communities and *Synechococcus* populations were calculated

there was a 4-fold reduction in nitrate concentrations in the upper 25 m of the water-column (417 µmol NO₃ m⁻²) and a *Synechococcus* bloom occurred. Elevated surface nitrate concentrations on 20 July (Fig. 3) were accompanied by high rates of primary production (Table 2), and were followed 48 h later by a 50 % net increase in *Synechococcus* cell numbers (Fig. 4). In contrast, numbers of ultraplankton algae remained the same (Fig. 4). Numbers of *Synechococcus* spp. subsequently declined from a maximum of 17×10^6 cells l⁻¹ in surface waters (2 to 5 m) to 8×10^6 cells l⁻¹ by 25 July. Between 20 and 25 July, the 4-fold reduction in nitrate concentrations in the upper 25 m of the water-column was accompanied by a 5-fold decrease in PE concentrations, but only a 2-fold decrease in Chl concentrations (Fig. 3). The reduction in PE concentrations in surface waters was not solely due to the decrease in *Synechococcus* cell numbers, since the mean cellular PE content also declined from 24 to 6 fg PE cell⁻¹ during the same period.

Temporal changes in *Synechococcus* abundance were not confined to the surface isothermal layer, since cell numbers doubled between 20 and 22 July at the Chl maximum (Fig. 4). After 22 July, *Synechococcus* cell numbers declined and the Chl maximum became deeper and more sharply defined. By 25 July, PE concentrations below the 7 % light level were also 2 to 4

times lower than those at a corresponding depth 5 d earlier (Fig. 3).

Depth-dependent differences between stations

Despite the temporal variability at Stn 1, differences were still evident in phytoplankton distributions between the 2 stations. Comparing time course data sets obtained over 5 d at each station, we found that *Synechococcus* spp. were consistently more abundant within the surface isothermal layer, the PE/nitracline maximum and the Chl maximum at Stn 1 (Fig. 4). The higher phytoplankton abundance presumably explains why the attenuation coefficient for the water-column was higher at Stn 1 (Fig. 2). While numbers of ultraplankton algae increased below the nitracline at both stations, they were nearly twice as abundant at the Chl maximum and 0.5 % light level of Stn 2, where they represented 54 to 75 % of all autofluorescent ultraplankton (<5 µm) cells (Fig. 4). Station differences in the relative abundance of the 2 ultraphytoplankton components were also reflected in estimates of their separate contributions to the standing crop of Chl. Fig. 7 shows that *Synechococcus* spp. made a greater contribution to integrated water-column Chl at Stn 1, compared with that at Stn 2, while the converse was true for ultraplankton algae.

Fig. 8. Distribution of *Synechococcus* spp. in 3 size fractions (black: 0.2 to 0.6 µm; hatched: 0.6 to 1 µm; stippled: 1 to 5 µm), at 4 locations in the water-column over the course of the monitoring periods at Stn 1 (left graph) and Stn 2 (right graph)

Fig. 9. Percentage of *Synechococcus* cells labelled by antiserum directed against the Sargasso Sea isolate WH7803 in 2 size fractions at different light depths on 20 July at Stn 1 (left graph) and on 2 August at Stn 2 (right graph)

Not only were ultraplankton algae more abundant below the 10% light level at Stn 2 (Fig. 5), but there were proportionately more smaller cells (Fig. 10). There was a reduction in mean cell size below the nitracline, and 79 to 89% of the algae at the base of the photic layer at Stn 2 were in the picoplankton fraction (passing through 1 µm Nuclepore filters).

DISCUSSION

Open ocean regions constitute three-quarters of the world's oceans, yet the rates of oceanic carbon and nitrogen cycling are uncertain by one order of magnitude (e.g. Eppley 1980, Jenkins & Goldman 1985). Some of the discrepancies between data sets may be

due to the time scales over which observations were made (Kerr 1986). It has been suggested that in nitrogen-limited ocean systems, episodic increases in nitrate concentrations may support a significant fraction of the total annual primary production (Platt & Harrison 1985). Nitrate pulses could be easily missed by occasional sampling strategies. Results from Stn 1 show that transient nanomolar increases in surface nitrate concentrations do indeed occur in stratified oceanic waters over a few days (Fig. 3), and can rapidly enhance phytoplankton production over similar time scales (Fig. 4). The temporal sequence of phytoplankton properties describing the nitrate-dependent *Synechococcus* bloom in the surface isothermal layer at Stn 1 is provided in more detail in a note by Glover et al. (1988). The change in nitrate concentrations between 20 and 25 July was stoichiometrically consistent with the subsequent cellular production of *Synechococcus* carbon and measured grazing rates (Glover et al. 1988). Data suggested that *Synechococcus* spp. alone responded

to the enhanced supply of nitrate, which may have been due to faster uptake kinetics conferred by their high surface area/volume ratio (Raven 1986), but may also reflect a capacity to rapidly translate increased cellular nitrogen into growth and cell division (Collos 1986).

The source of the initially high nitrate concentrations in the surface isothermal layer is a matter of conjecture. A numerical study showed that wind-surface current interactions can affect both the instantaneous nutrient distribution and the mean level of its transport through the thermocline (Klein & Coste 1984), and there were major storms in the Sargasso Sea between 2 and 4 July and between 9 and 11 July (log of RV 'Cape Hatteras' Cruise CH-12-86). These events were apparently sufficient to cause mixing at Stn 1, which resulted in a surface isothermal layer that was 1°C cooler than typical seasonal values, and temperatures of >26°C were observed only after 23 July (Fig. 2). Using the chemiluminescent nitrate method, Eppley & Renger (1988) also reported nanomolar increases in surface layer nitrate concen-

Fig. 10. Distribution of ultraplankton algae in 3 size fractions (black: 0.2 to 0.6 μm; hatched: 0.6 to 1 μm; stippled: 1 to 5 μm), at 4 locations in the water-column over the course of the monitoring periods at Stn 1 (left graph) and Stn 2 (right graph)

trations following a small wind event in stratified California waters.

It is well established that oceanic *Synechococcus* spp. use PE as their primary light-harvesting pigment (see review by Glover 1985), but the ability of marine *Synechococcus* spp. to use some of their PE pigment as a nitrogen reserve has recently been debated (Barlow & Alberte 1985, Wyman et al. 1985, Glibert et al. 1986, Yeh et al. 1986 vs Wyman et al. 1986, Kana & Glibert 1987). Data presented here support the additional role of PE in nitrogen storage: first, the 4-fold decline in surface layer nitrate concentrations between 20 and 25 July at Stn 1 (Fig. 3) was accompanied by a 4-fold decrease in PE concentrations within *Synechococcus* cells (24 to 6 fg PE cell⁻¹). Secondly, in the surface isothermal layer there was a 2-fold decline in PE/Chl ratios in the total *Synechococcus* population (>0.6 µm) during this period (Glover et al. 1988). This change in the pigment ratio of *Synechococcus* cells is unlikely to have resulted from photoadaptation, since populations were at light-saturating depths for growth 90 to 34 % I₀ (Glover et al. 1987), with I_k values (Table 2) and Chl contents (Fig. 6) that are typical of high-light adapted cells (Glibert et al. 1986, Prézélin et al. 1986, Glover et al. 1987). Thus, our data suggest a causal relationship between initially high nitrate concentration and high cellular PE content.

Our data provide the first measurements of maxima in PE concentrations per liter in the Sargasso Sea by looking at glycerol-uncoupled PE fluorescence yield. Without these measurements, we would not have had a sampling rationale for locating sites of PE-rich *Synechococcus* cells with high photosynthetic activity, nor would we have been able to determine how the PE content of *Synechococcus* cells varied with depth-dependent changes in light and nutrient availability. The deep PE maxima we identified were associated with the nitracline (Fig. 3) and resulted from the increased PE content of *Synechococcus* cells and not from an increase in their abundance (compare Figs. 5 and 6). These data substantiate earlier flow cytometry results of an increase in PE fluorescence intensity per cell with increasing depth (Olson et al. 1985). While *Synechococcus* cells had a relatively high PE content at deep PE maxima (Fig. 6), I_k values attested to the fact that communities would not be limited by the in situ irradiance during most of the day and primary productivity was relatively high (Table 2). Thus, these *Synechococcus* populations appeared to effectively use the increased nitrogen flux at the nitracline for PE synthesis, thereby enhancing absorption of the available blue radiation and resulting in relatively high cellular rates of photosynthesis. Furthermore, these *Synechococcus* populations were not dominated by the WH7803 serotype (Fig. 9), but contained cells whose

PE fluorescence emission characteristics were typical of clone WH8103 (Campbell & Iturriaga 1988), whose higher complement of phycourobilin to phycoerythrobilin chromophores enhances absorption at shorter blue wavelengths of 490 nm (Ong et al. 1984). Using flow cytometry single cell analysis, Olson et al. (1988) reported that the high phycourobilin pigment type invariably dominated oceanic *Synechococcus* populations from other regions.

Comparing our results with data sets from several other investigations (Glover et al. 1985, Murphy & Haugen 1985, Waterbury et al. 1986, Campbell & Carpenter 1987, Iturriaga & Marra 1988), it appears that there is considerable temporal and spatial variability in the abundance and composition of Sargasso Sea *Synechococcus* populations: Fig. 3 demonstrates the variability that we observed in PE concentrations per liter above the thermoclines (>30 % I₀) at the 2 stations. These differences were not solely related to *Synechococcus* cellular abundance. The 10 m transient PE maximum at Stn 1 was due to a *Synechococcus* population with increased pigment content (28 fg PE cell⁻¹) and relatively high photosynthetic activity (Table 2). These results suggest that this 10 m community was actively growing and that the isothermal layer was not necessarily a homogenous mixed layer at this time. *Synechococcus* cell numbers at Stn 1 were always higher in surface waters, the PE maximum and the Chl maximum, compared with those at Stn 2 (Fig. 4). This was reflected in their contribution to the standing crop of Chl (Fig. 7) and the different attenuation coefficients of the 2 water-columns (Fig. 2). The significance of *Synechococcus* spp. at the Chl maximum of Stn 1 was shown by their enhanced contribution to the Chl concentration (Fig. 7) and a doubling in their abundance (Fig. 4) over the 3 d in which the temporal changes occurred in the upper 25 m.

Reported abundances of *Synechococcus* spp. in July and August in Sargasso surface waters range from 3 to 17 × 10⁶ cells l⁻¹ (Glover et al. 1985, Murphy & Haugen 1985, Waterbury et al. 1986) with the maximum value being found on 22 July at Stn 1 (Fig. 4). In stratified oceanic regions, greatest numbers of *Synechococcus* spp. are often in surface waters (Waterbury et al. 1986; Fig. 4), but a maximum has been found at much lower light levels, where the nutrient supply is increased (Glover et al. 1985, Murphy & Haugen 1985, Waterbury et al. 1986, Campbell & Carpenter 1987). In April 1985, Iturriaga & Marra (1988) recorded maximum abundances of *Synechococcus* cells in surface waters north of the Subtropical Atlantic Convergence, while maximum numbers occurred near the bottom of the euphotic zone south of the front (24 °N). Variability also occurs in the serogroup composition of *Synechococcus* spp.

populations in the Sargasso Sea (Campbell & Carpenter 1987).

While *Synechococcus* spp. were the dominant component of ultraphytoplankton communities above 10 % I_0 , Chl-fluorescing algae became the dominant component of ultraplankton biomass at the bottom of the euphotic zone, particularly at Stn 2 (Figs. 4, 5 and 7). Data from Stn 2 (Figs. 4 and 5) confirm previous light depth distribution patterns in the composition of ultraplankton in stratified oceanic systems (Furuya & Marumo 1983, Takahashi & Bienfang 1983, Takahashi & Hori 1984, Glover et al. 1985, Murphy & Haugen 1985, Gieskes & Kraay 1986, Trees et al. 1986).

At both stations there was a change in the cell size distribution of ultraplankton algal communities with increasing depth (Fig. 10). The majority of algae were in the 1 to 5 μm fraction above the nitracline, while with increasing depth below it, there were proportionately more smaller cells. This was particularly evident at Stn 2, where 79 to 89 % of algal cells at the bottom of the euphotic layer were in the < 1 μm picoplankton fraction (Fig. 10). Since sinking rates of these small forms is negligible (Takahashi & Bienfang 1983), increases in the abundance of picoplankton algae at depth (Fig. 5) may be attributed to in situ growth, and their ecological success at low intensities of blue-violet light may be due, in part, to their complement of blue-violet absorbing accessory pigments (Brown 1985, Wood 1985, Glover et al. 1987) and a relatively high specific absorption coefficient conferred by their small cell volume (Kirk 1986). While the high phycourobilin to phycoerythrobilin chromophore complement in the PE of *Synechococcus* populations would enhance their ability to use the blue light available at the nitracline PE maximum, the pigmentation of oceanic picoplankton algae gives them a competitive advantage deeper in the water-column. It should be noted however, that while the numerical abundance of ultraplankton algae was greatest in the blue-violet light regime found at 0.5 % I_0 at both stations (Fig. 5), low I_k values and photosynthetic rates (Table 2) suggested that even though these communities were shade-adapted, photosynthesis was light-limited.

The numerical dominance of *Synechococcus* spp. in surface waters (Fig. 4) cannot be explained in terms of irradiance, since at saturating intensities of white light and nutrient sufficient conditions, clones of oceanic *Synechococcus* and ultraplankton algae have comparable growth rates (Glover et al. 1987). Consequently, the varying depth distributions of the 2 ultraplankton groups can be partially explained in terms of light intensity and quality, but it seems likely that differences in nutrient uptake kinetics could also be a factor.

In conclusion, comparisons between previous data sets and those presented here for summer Sargasso Sea

communities suggest that there is a considerable degree of temporal and geographic variability in the abundance and composition of ultra- and pico-phytoplankton at comparable locations within the water-column.

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LITERATURE CITED

- Barlow, R. G., Alberte, R. S. (1985). Photosynthetic characteristics of phycoerythrin-containing marine *Synechococcus* spp. I. Responses to growth photon flux density. *Mar. Biol.* 86: 63–74
- Brown, J. S. (1985). Three photosynthetic antenna porphyrins in a primitive green alga. *Biochim. Biophys. Acta* 807: 143–146
- Campbell, L., Carpenter, E. J. (1987). Characterization of phycoerythrin-containing *Synechococcus* spp. populations by immunofluorescence. *J. Plankton Res.* 9: 1167–1181
- Campbell, L., Iturriaga, R. (1988). Identification of *Synechococcus* spp. in the Sargasso Sea by immunofluorescence and fluorescence excitation spectroscopy performed on individual cells. *Limnol. Oceanogr.* (in press)
- Collos, Y. (1986). Time-lag algal growth dynamics: biological constraints on primary production in aquatic environments. *Mar. Ecol. Prog. Ser.* 33: 193–206
- Epplé, R. W. (1980). Estimating phytoplankton growth rates in the central oligotrophic oceans. In: Falkowski, P. G. (ed.) *Primary productivity in the sea*. Brookhaven Symposia in Biology, Vol. 31. Plenum Press, New York, p. 231–242
- Epplé, R. W., Renger, E. H. (1988). Nanomolar increases in surface layer nitrate concentration following a small wind event. *EOS* 68: Abstract 12D–11
- Fitzwater, S. E., Knauer, G. A., Martin, J. H. (1982). Metal contamination and its effect on primary production measurements. *Limnol. Oceanogr.* 27: 544–551
- Fogg, G. E. (1986). Picoplankton. *Proc. R. Soc. Lond. B* 228: 1–30
- Furuya, K., Marumo, R. (1983). The structure of the phytoplankton community in the subsurface chlorophyll maximum in the western North Pacific. *J. Plankton Res.* 5: 393–406
- Garside, C. (1982). A chemiluminescent technique for the determination of nanomolar concentrations of nitrate, nitrite and nitrite, or nitrite alone in seawater. *Mar. Chem.* 11: 159–167
- Gieskes, W. W. C., Kraay, G. W. (1986). Floristic and physiological differences between the shallow and deep nanophytoplankton community in the euphotic zone of the open tropical Atlantic revealed by HPLC analysis of pigments. *Mar. Biol.* 91: 567–576
- Glibert, P. M., Kana, T. M., Olson, R. J., Kirchman, D. L., Alberte, R. S. (1986). Clonal comparisons of growth and photosynthetic responses to nitrogen availability in marine *Synechococcus* spp. *J. exp. mar. Biol. Ecol.* 101: 199–208

- Glover, H. E. (1985). The physiology and ecology of the marine cyanobacterial genus *Synechococcus*. In: Jannasch, H. W., Williams, P. J. LeB. (eds.) *Advances in aquatic microbiology*. Academic Press, London, p. 49–107.
- Glover, H. E., Smith, A. E., Shapiro, L. P. (1985). Diurnal variations in photosynthetic rates: comparisons of ultraphytoplankton with a larger size fraction. *J. Plankton Res.* 7: 519–535.
- Glover, H. E., Campbell, L., Prézélin, B. B. (1986). Contribution of *Synechococcus* to size-fractionated primary productivity in three water masses in the Northwest Atlantic Ocean. *Mar. Biol.* 91: 193–203.
- Glover, H. E., Keller, M. D., Spinrad, R. W. (1987). The effects of light quality and intensity on photosynthesis and growth of marine eukaryotic and prokaryotic phytoplankton clones. *J. exp. mar. Biol. Ecol.* 105: 137–159.
- Glover, H. E., Prézélin, B. B., Campbell, L., Wyman, M., Garside, C. (1988). Observations of a nitrate-dependent *Synechococcus* bloom in surface Sargasso Sea water. *Nature, Lond.* 331: 161–163.
- Hobbie, J. E., Daley, R. J., Jasper, S. (1977). Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. environ. Microbiol.* 33: 1225–1228.
- Iturriaga, R., Marra, J. (1988). Temporal and spatial variability of chroococcoid cyanobacteria, *Synechococcus* spp., specific growth rates and their contribution to primary production in the Sargasso Sea. *Mar. Ecol. Prog. Ser.* 44: 175–181.
- Jenkins, W. J., Goldman, J. C. (1985). Seasonal oxygen cycling and primary production in the Sargasso Sea. *J. mar. Res.* 43: 465–491.
- Kana, T. M., Glibert, P. M. (1987). Effect of irradiances up to 2000 $\mu\text{E m}^{-2} \text{s}^{-1}$ on marine *Synechococcus* WH7803 – I. Growth, pigmentation and cell composition. *Deep Sea Res.* 34: 479–495.
- Kerr, R. A. (1986). The ocean's deserts are blooming. *Science* 232: 1345.
- Kirk, J. T. O. (1986). Optical properties of picoplankton suspensions. In: Platt, T., Li, W. K. W. (eds.) *Photosynthetic picoplankton*. Can. Bull. Fish. Aquat. Sci. 214: 501–520.
- Klein, P., Coste, B. (1984). Effects of wind-stress variability on nutrient transport into the mixed layer. *Deep Sea Res.* 31: 21–37.
- Laws, E. A., DiTullio, G. R., Redalje, D. G. (1987). High phytoplankton growth and production rates in the North Pacific subtropical gyre. *Limnol. Oceanogr.* 32: 905–918.
- Marra, J., Heinemann, K. R. (1987). Primary production in the North Pacific Central Gyre: some measurements based on ^{14}C . *Deep Sea Res.* 34: 1821–1829.
- Murphy, L. S., Haugen, E. M. (1985). The distribution and abundance of phototrophic ultraplankton in the North Atlantic. *Limnol. Oceanogr.* 30: 47–58.
- Olson, R. J., Vulot, D., Chisholm, S. W. (1985). Marine phytoplankton distributions measured using shipboard flow cytometry. *Deep Sea Res.* 32: 1273–1280.
- Olson, R. J., Chisholm, S. W., Zettler, E. R., Ambrust, E. V. (1988). Analysis of *Synechococcus* pigment types in the sea using single and dual beam flow cytometry. *Deep Sea Res.* 34: 425–440.
- Ong, L. J., Glazer, A. N., Waterbury, J. B. (1984). An unusual phycoerythrin from a marine cyanobacterium. *Science* 224: 80–83.
- Platt, T., Harrison, W. G. (1985). Biogenic fluxes of carbon and oxygen in the ocean. *Nature, Lond.* 318: 55–58.
- Platt, T., Li, W. K. W. (1986). Photosynthetic picoplankton. *Can. Bull. Fish. Aquat. Sci.* 214: 1–583.
- Prézélin, B. B., Bidigare, R. R., Matlick, H. A., Putt, M., Verhoven, B. (1987). Diurnal patterns of size-fractionated primary productivity across a coastal front. *Mar. Biol.* 96: 563–574.
- Prézélin, B. B., Putt, M., Glover, H. E. (1986). Diurnal patterns in photosynthetic capacity and depth-dependent photosynthesis-irradiance relationships in *Synechococcus* spp. and larger phytoplankton in three water masses in the Northwest Atlantic Ocean. *Mar. Biol.* 91: 205–217.
- Raven, J. A. (1986). Physiological consequences of extremely small size for autotrophic organisms in the sea. In: Platt, T., Li, W. K. W. (eds.) *Photosynthetic picoplankton*. Can. Bull. Fish. Aquat. Sci. 214: 1–70.
- Stockner, J. G., Antia, N. J. (1986). Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. *Can. J. Fish. Aquat. Sci.* 43: 2472–2503.
- Strickland, J. D. H., Parsons, T. R. (1972). A practical handbook of seawater analysis, 2nd edn. *Bull. Fish. Res. Bd Can.* 167.
- Takahashi, M., Bienfang, P. K. (1983). Size structure of phytoplankton biomass and photosynthesis in subtropical and tropical Hawaiian waters. *Mar. Biol.* 76: 203–211.
- Takahashi, M., Hori, T. (1984). Abundance of picophytoplankton in the subsurface chlorophyll maximum layer in subtropical and tropical waters. *Mar. Biol.* 79: 177–186.
- Trees, C. C., Bidigare, R. R., Brooks, J. M. (1986). Distribution of chlorophylls and phaeopigments in the Northwest Atlantic Ocean. *J. Plankton Res.* 8: 447–458.
- Waterbury, J. B., Watson, S. W., Valois, F. W., Franks, D. G. (1986). Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. In: Platt, T., Li, W. K. W. (eds.) *Photosynthetic picoplankton*. Can. Bull. Fish. Aquat. Sci. 214: 71–120.
- Wood, A. M. (1985). Adaptation of photosynthetic apparatus of marine ultraphytoplankton to natural light fields. *Nature, Lond.* 316: 253–255.
- Wyman, M., Gregory, R. P. F., Carr, N. G. (1985). Novel role for phycoerythrin in a marine cyanobacterium, *Synechococcus* strain DC-2. *Science* 230: 818–820.
- Wyman, M., Gregory, R. P. F., Carr, N. G. (1986). Role of phycoerythrin in marine *Synechococcus* spp. *Science* 234: 1423–1424.
- Wyman, M., Carr, N. G. (1988). An *in vivo* method for the estimation of phycoerythrin concentrations in marine cyanobacteria (*Synechococcus* spp.). *Limnol. Oceanogr.* (in press).
- Yeh, S. W., Ong, L., Glazer, A. N. (1986). Role of phycoerythrin in marine picoplankton *Synechococcus* spp. *Science* 234: 1422–1423.