

RAPID RESPONSE PAPER

Effect of irradiances up to $2000 \mu\text{E m}^{-2} \text{s}^{-1}$ on marine *Synechococcus* WH7803—I. Growth, pigmentation, and cell composition

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Abstract—We grew *Synechococcus* WH7803 at rates exceeding 1.4 d^{-1} at irradiances from 200 to $2000 \mu\text{E m}^{-2} \text{s}^{-1}$ under continuous light in nutrient replete media with no evidence of photoinhibition. Concentrations of the photosynthetic pigments phycoerythrin, phycocyanin, and chlorophyll *a*, were inversely related to growth irradiance. Phycoerythrin exhibited the greatest plasticity with the concentration in cells adapted to $30 \mu\text{E m}^{-2} \text{s}^{-1}$ being *ca.* 20 times greater than that in cells adapted to $700 \mu\text{E m}^{-2} \text{s}^{-1}$. Changes in the phycoerythrin:phycocyanin ratio as well as their respective concentrations indicate that phycobilisomes underwent changes in size at irradiances which saturated or nearly saturated growth and underwent changes in number at irradiances which limited growth. Phycoerythrin in high light adapted cells contained <3% of the cell nitrogen as opposed to >20% in light limited cells. Results support the notion that nutrient replete *Synechococcus* have the capacity to grow at maximal growth rates in brightly lit oceanic surface mixed layers.

INTRODUCTION

CHROOCOCCOID cyanobacteria of the genus *Synechococcus* have been shown to be important components of marine picoplankton in seas that span a diversity of light, temperature, and nutrient regimes. Interest in the ecology and physiology of these organisms stems from their cosmopolitan distribution (JOHNSON and SIEBURTH, 1979; WATERBURY *et al.*, 1979; MURPHY and HAUGEN, 1985), often high volume-based rates of productivity relative to other phytoplankton (PLATT *et al.*, 1983; LI *et al.*, 1983), especially in oligotrophic regions (GLOVER *et al.*, 1985), and spectral characteristics that distinguish them from most eukaryotic phytoplankton (ALBERTE *et al.*, 1984), and which make them highly suitable for measurement by remote sensing methodologies (EXTON *et al.*, 1983). Much emphasis over the past several years has been placed on determining the photosynthetic and growth response of these organisms to irradiance in an effort to understand how light availability may determine the distribution patterns of these organisms (e.g. MORRIS and GLOVER, 1981; GLOVER and MORRIS, 1981; PLATT *et al.*, 1983; BARLOW and ALBERTE, 1985). The prevailing view (cf. GLOVER, 1986; FOGG, 1986)

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is that *Synechococcus* spp. are adapted to "low" light intensities and grow best deep in the euphotic zone.

This conclusion has been based on interpretations of results from both laboratory and field investigations. The evidence includes: (a) observations of subsurface maxima in cell density (MURPHY and HAUGEN, 1985), (b) possession of the photosynthetic pigment-protein phycoerythrin (PE), which absorbs green light maximally and therefore exploits the more deeply penetrating light in the water column (WOOD, 1985; GLOVER *et al.*, 1986), (c) more efficient utilization of "white" light for growth and photosynthesis relative to other phytoplankton classes (MORRIS and GLOVER, 1981; BARLOW and ALBERTE, 1985), (d) the tendency to exhibit inhibition of growth and photosynthesis at moderate to high irradiances (PLATT *et al.*, 1983; BARLOW and ALBERTE, 1985) and (e) measurements of low photosynthetic capacity in surface populations relative to those at depth (GLOVER *et al.*, 1985). Specifically, MORRIS and GLOVER (1981) reported that growth of *Synechococcus* DC-2 (now called WH7803) saturated at $45 \mu\text{E m}^{-2} \text{s}^{-1}$ and remained maximal up to $70 \mu\text{E m}^{-2} \text{s}^{-1}$ (their highest experimental irradiance), and BARLOW and ALBERTE (1985) showed that *Synechococcus* WH7803 as well as clone WH8018 (previously called L1604) became light saturated for growth at irradiances between 25 and $50 \mu\text{E m}^{-2} \text{s}^{-1}$ and was severely inhibited (i.e. no growth) at $250 \mu\text{E m}^{-2} \text{s}^{-1}$.

However, there have been several recent field studies that have indicated that apparently vigorous ($\mu > 0.6 \text{ d}^{-1}$) *Synechococcus* populations occur in surface mixed layers (LANDRY *et al.*, 1984; ITURRIAGA and MITCHELL, 1986; GIESKES and KRAAY, 1986; CAMPBELL and CARPENTER, 1986), suggesting that growth, in nature, may not be inhibited by high irradiance. GIESKES and KRAAY (1986), studying the eastern tropical Atlantic Ocean, reported rapid ^{14}C incorporation and high specific growth rates (by the method of REDALJE and LAWS, 1981) in surface mixed layer assemblages dominated by *Synechococcus*. ITURRIAGA and MITCHELL (1986) estimated comparable growth rates for *Synechococcus* growing in the surface mixed layer and at a depth of 75 m at an oceanic station in the North Pacific, and LANDRY *et al.* (1984) estimated growth rates of 1.4–2.0 d^{-1} for natural samples suspended at a depth of 5 m in Kaneohe Bay, Hawaii. CAMPBELL and CARPENTER (1986) used the frequency of dividing cell technique to estimate growth rates of natural populations of *Synechococcus* and concluded that samples collected at the surface or at depth generally grew faster when incubated at surface irradiances. These observations are also supported by the lack of photoinhibition of photosynthesis in *Synechococcus* populations growing in the surface mixed layer (GLOVER *et al.*, 1985; ITURRIAGA and MITCHELL, 1986; JOINT and POMROY, 1986; PRÉZELIN *et al.*, 1986). Thus, there are significant discrepancies between results of previous laboratory and field studies in terms of the irradiance response of *Synechococcus*.

A second physiological characteristic that has been recently described relates to the apparently unique way in which marine *Synechococcus* regulates cellular nitrogen. It is often stated that in cyanobacteria, phycobiliproteins, the major photosynthetic accessory pigments, comprise 40–50% of the soluble cell protein under optimal growth conditions (e.g. MYERS and KRATZ, 1955; BENNETT and BOGORAD, 1973; GLAZER, 1981), and thus represent a major portion of total cellular nitrogen. The mobility or breakdown of this pool, it can be argued, might be of some advantage to a cell during periods of nitrogen impoverishment, in that the components (amino acids) can be used to support growth and maintenance.

The effect of nitrogen starvation on cyanobacteria has been studied most extensively in the fresh water species, *Anacystis nidulans*. When starved of nitrogen, these cells terminate phycobiliprotein synthesis (LAU *et al.*, 1977) and degrade the already present phycobilisomes (YAMANAKA and GLAZER, 1980). The specific suggestion has been made that degradation of accessory pigments is a mechanism to mobilize amino acids for growth functions during periods of nitrogen deprivation (PERRY *et al.*, 1981). This seems reasonable because nitrogen-starved cells lacking phycobilisomes maintain some capacity to photosynthesize by virtue of their retained chlorophyll (LEMASSON *et al.*, 1973).

A wrinkle in this scheme was introduced by KURSAR *et al.* (1981) when they reported that only 8% of the PE in *Synechococcus* WH7803 sedimented with the phycobilisome fraction in sucrose gradients. They attributed this to "unusual instability" of the phycobilisomes, but, in interpreting the high PE fluorescence from these cells, they suggested that a "free" pool may actually exist *in vivo*. Thus was born the idea that *Synechococcus* WH7803 possesses two significant fractions of PE, one which is bound to the phycobilisome and one which is uncoupled to photosynthesis or "free". BARLOW and ALBERTE (1985), in attempting to explain their unusual finding of increased photosynthetic unit (PSU) size with increased growth irradiance, suggested that the presence of the suspected free PE pool would make it only appear as though the functional PSUs were increasing. They were the first to make the seemingly logical suggestion that the free PE served independently as a nitrogen storage pool by virtue of its high amino-nitrogen content. The first study purporting to directly demonstrate the presence of the free PE pool *in vivo* in clone WH7803, as well as its functional role, was that of WYMAN *et al.* (1985). In reviewing this issue recently, FOGG (1986) suggested that PE may actually be "more important as a nitrogen reserve" than as a light harvesting compound.

In direct contrast with both of the prevailing notions concerning the physiological adaptations of marine *Synechococcus* WH7803 to light and nitrogen, we have been able to obtain high growth rates of this clone at irradiances up to 2000 $\mu\text{E m}^{-2} \text{s}^{-1}$ in the laboratory with no evidence of photoinhibition of growth or photosynthesis, and have found that in rapidly growing cells, PE concentration may be <5% of that in light-limited cells, effectively minimizing the storage role of this molecule. In this paper, we describe the growth and pigmentation response, and in the following paper the photosynthetic response, of *Synechococcus* WH7803 adapted to scalar irradiances between 30 and 2000 $\mu\text{E m}^{-2} \text{s}^{-1}$. These findings necessitate a re-evaluation of the capacity of *Synechococcus* to grow rapidly at irradiances found in near-surface waters and the universality of the previously held notions concerning this organism's response to light and nitrogen.

METHODS

Growth

Synechococcus WH7803 was pre-adapted to each of eight irradiances for >2 weeks prior to experimentation. Maintenance stocks, which had been growing under ambient room light, were stepped up to the experimental irradiances by allowing a 3–4 day adjustment period after each moderate (2–3 fold) increase in irradiance. Working stocks were then maintained in a light gradient for 2 weeks to several months by frequent (every 3–5 d) transfers of dilute batch cultures. Continuous light was provided by a bank of cool-white fluorescent tubes. The highest irradiances were obtained by placing mirrors behind

the culture vessels (600 ml Corning polystyrene tissue culture flasks). Culture medium was F/4 (-Si, -Cu, -vitamins) (GUILLARD and RYTHER, 1962). A blower assisted in maintaining cultures at room temperature (22–23°C), although the temperature of the cultures at 700, 1330, and 2000 $\mu\text{E m}^{-2} \text{s}^{-1}$ was elevated above room temperature 1, 1.5, and 3°C, respectively.

Quantum scalar irradiances were measured with a recently factory-calibrated Biospherical Instruments QSL-100 photosynthetically active radiation (PAR) irradiance meter with 4π sensor. Measurements were made dry in the position of the culture vessels. Values obtained with the probe inside the vessel were elevated due to internal reflection off the probe and walls, and therefore did not reliably measure *in situ* irradiance. Variation in irradiance over the front surface of the vessel was generally $< \pm 10\%$ of the mid-point reading. No correction was made for attenuation by the walls of the vessels or for internal reflection. Photon flux densities were measured for comparison using a LiCor Li-185B UWQ PAR meter with a cosine-corrected 2π sensor by summing forward and backward readings. In our experimental light field, which was relatively diffuse, the corresponding values were highly correlated ($r^2 = 0.996$; $n = 8$) with scalar irradiance equaling 3.0 times photon flux density.

Experiments were initiated by inoculating exponentially growing cultures in fresh media to a cell density of *ca.* 0.5×10^6 cells ml^{-1} . Flasks were shaken by hand at frequent (every 2–4 h) intervals. Growth rates were determined from cell counts 2–3 times daily. Cell counts were made by filtering aliquots onto 0.2 μm Nuclepore filters, oiling the filters, and counting autofluorescent cells through a $\times 100$ oil objective. Cultures were harvested when they reached a density between 6×10^6 and 15×10^6 cells ml^{-1} . The cultures were only weakly turbid at these final cell densities. All pigment, carbon (C) and nitrogen (N) analyses were conducted on replicated cultures, but growth rates were determined only for a single culture at each growth irradiance.

Cell composition and size

Cells were harvested by filtration and stored in liquid nitrogen either on filters or in 0.1 M potassium phosphate buffer (pH 7). Chlorophyll *a* (Chl *a*) was determined by filtering cells onto Whatman GF/F filters, extracting in 90% acetone under dim light and quantifying by reverse phase HPLC according to MANTOURA and LEWELLYN (1983) and using chromatographically purified Chl *a* as a standard for calibration. Cells were concentrated for determinations of phycocyanin (PC) and phycoerythrin (PE) by washing filtered cells off 0.4 μm Millipore filters with buffer. Cell yield by this technique was $>99\%$. The concentrated cells were passed 3 times through a French pressure cell at 24,000 psi, followed by centrifugation at 110,000 *g* (Spinco model L; Type 40 rotor at 35,000 rpm) for 1 h to remove debris and membranes. Absorbance was measured by a Bausch and Lomb Spectronic 2000, and concentrations calculated according to BARLOW and ALBERTE (1985). Cell C and N concentrations were determined using a Perkin Elmer 240 elemental analyzer after filtration onto precombusted (550°C for 1 h) Whatman GF/F filters.

Cell dimensions were measured on >120 cells from single cultures from each of the growth irradiances. These particular measurements were made on cultures grown only for this purpose. Cells were fixed in 0.5% glutaraldehyde, placed on slides coated with a thin layer of agar and photographed under oil ($\times 1000$ magnification) using bright-field optics. Cell lengths and widths were determined using a digitizing table with resolution of

0.025 mm. Combined with photographic image resolution, statistical error (95% confidence interval) in measuring one cell 10 times was $< \pm 5\%$ of the mean. Volume was calculated using an algorithm based on the assumption that the cell shape was a cylinder with hemispherical ends.

Electron microscopy

Cells were prefixed in 0.5% glutaraldehyde (pH 6.8), fixed in 3.5% glutaraldehyde, and postfixed in 1.0% osmium followed by uranyl acetate. Fixed cells were dehydrated in an ethanol series and embedded in Spurr's plastic resin. Details of these procedures may be found in RIPPKA *et al.* (1974). Only cultures grown at 30, 160 and 1330 $\mu\text{E m}^{-2} \text{s}^{-1}$ were used for these observations.

Parameter calculations

Growth rate, μ , was calculated from the equation: $\mu = (\ln 2)/g$, where g is the generation time (days) determined from the slope of the growth curve. The maximum growth rate for these culture conditions, μ_{max} , was calculated from a linear transformation (Woelf plot) of the μ vs I curve. We excluded the 2000 $\mu\text{E m}^{-2} \text{s}^{-1}$ sample from the calculation of μ_{max} because it was likely influenced significantly by the elevated temperature of the culture. (Inclusion of this point elevates the estimate of μ_{max} by 0.15 d^{-1} .) The irradiance at which the μ vs I relationship saturated, I_{sat} , was taken from the intercept of the initial slope and μ_{max} lines from the μ vs $\log(\text{irradiance})$ plot.

RESULTS

Growth rate exhibited a saturation response with no indication of photoinhibition at irradiances up to 2000 $\mu\text{E m}^{-2} \text{s}^{-1}$ (Fig. 1). The irradiance at which growth first saturated

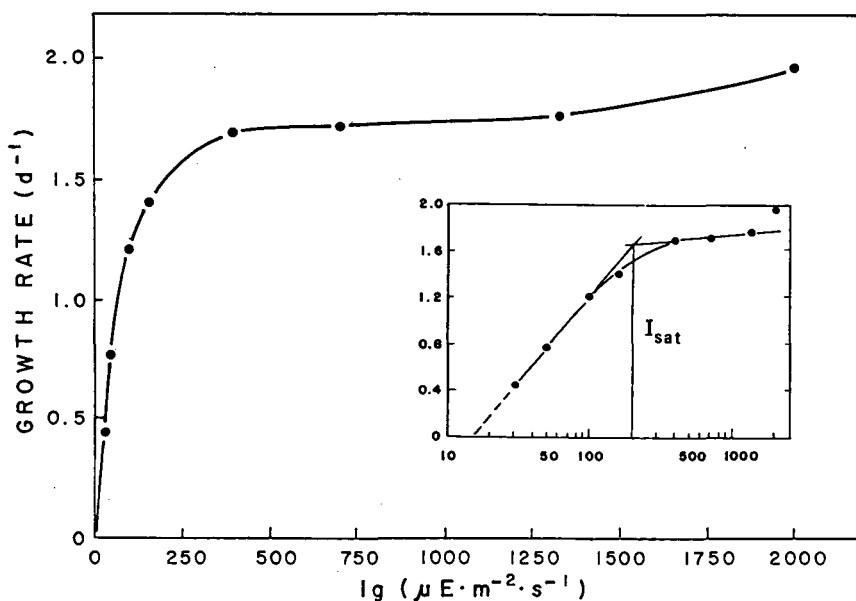


Fig. 1. The effect of irradiance on growth rate of *Synechococcus* WH7803. Inset shows the determination of the saturating irradiance (I_{sat}). Elevation of the growth rate at 2000 $\mu\text{E m}^{-2} \text{s}^{-1}$ was due to the higher temperature of that culture (see Methods).

(I_{sat}) was $200 \mu\text{E m}^{-2} \text{s}^{-1}$. Growth rates exceeded 1.7 d^{-1} at irradiances $>400 \mu\text{E m}^{-2} \text{s}^{-1}$, with $\mu_{\text{max}} = 1.87 \text{ d}^{-1}$ (=9 h generation time). Note that the growth rate at $2000 \mu\text{E m}^{-2} \text{s}^{-1}$ was elevated due to temperature effects, and was therefore not included in this calculation. Growth at limiting irradiances was a linear function of the log of irradiance between 30 and $100 \mu\text{E m}^{-2} \text{s}^{-1}$ (Fig. 1).

Cell size increased with decreasing growth irradiance (I_g) by parallel increases in cell length and width (Fig. 2). Cells grown at $30 \mu\text{E m}^{-2} \text{s}^{-1}$ were *ca.* 40% larger in volume than cells grown at $2000 \mu\text{E m}^{-2} \text{s}^{-1}$. Cell C varied from 200 to 300 fg cell^{-1} (the mean value was $250 \text{ fg C cell}^{-1}$), and was independent of I_g (Fig. 3A). Cell N ranged between 37 and 75 fg cell^{-1} and only showed a significant increase below $100 \mu\text{E m}^{-2} \text{s}^{-1}$. This is most clearly shown by its effect on the molar C:N ratio (Fig. 3B). This ratio increased from *ca.* 4.5 at $30 \mu\text{E m}^{-2} \text{s}^{-1}$ to 6.3 at irradiances exceeding I_{sat} . There appeared to be a decline in the ratio at the highest growth irradiances although there was considerable variability among replicate cultures.

An inverse relationship existed between I_g and PE, PC, and Chl *a* over most of the irradiance range tested (Figs 4A–C). Of the three pigments, PE exhibited the greatest plasticity with a >20 fold difference between highest and lowest concentrations (143 vs 6 fg cell^{-1}). The greatest change in PE concentration occurred at irradiances $<I_{\text{sat}}$, although a significant decrease in PE (on average, 45 down to 8 fg cell^{-1}) was also observed at irradiances $>I_{\text{sat}}$. At irradiances $>700 \mu\text{E m}^{-2} \text{s}^{-1}$, PE was at minimal concentration (6 – 9 fg cell^{-1}) in the cell. The large change in PE concentration accounted for the change in coloration of the cells from the typical red of phycoerythrin-rich cells observed at irradiances $<I_{\text{sat}}$ to a very pale yellow at the highest irradiances. The concentration of PC increased *ca.* 3 fold (3 – 10 fg cell^{-1}) from the highest to lowest growth irradiance. As with PE, the greatest change occurred at irradiances $<I_{\text{sat}}$, and

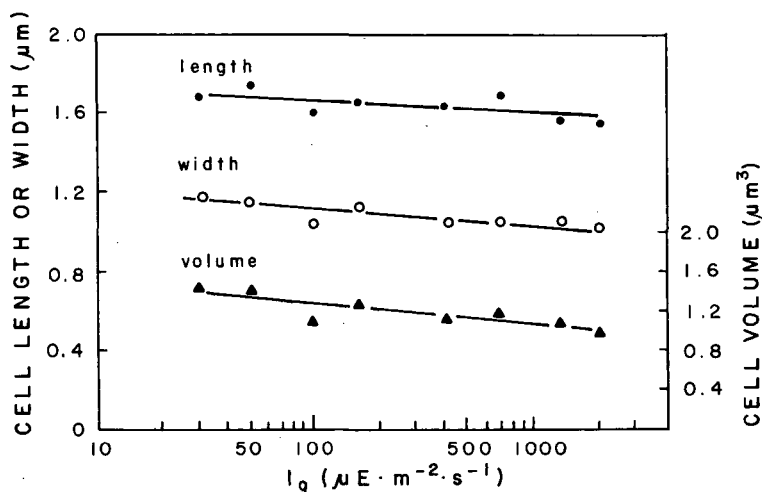


Fig. 2. Effect of growth irradiance on average cell length, width, and volume of *Synechococcus* WH7083. Ninety five percent confidence intervals for the mean generally do not extend beyond the size of the symbol.

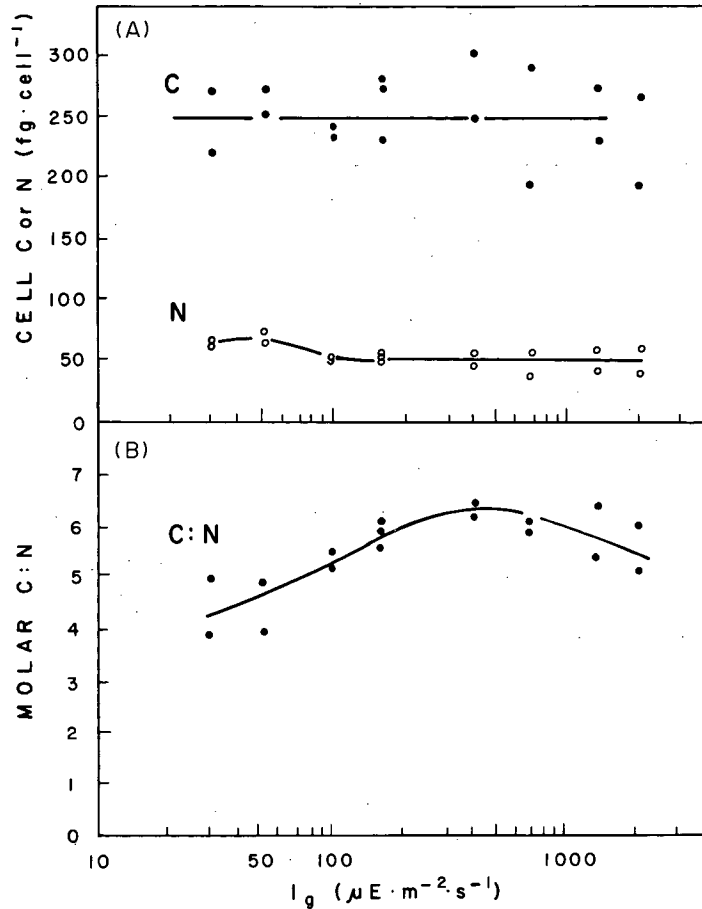


Fig. 3. The effect of growth irradiance on (A) cellular carbon and nitrogen concentrations, and (B) the carbon:nitrogen ratio expressed in moles: moles of *Synechococcus* WH7803.

minimal concentrations (*ca.* 3 $\text{fg} \cdot \text{cell}^{-1}$) were observed at irradiances $>700 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. The concentration of Chl *a* ranged from 1 to 5 $\text{fg} \cdot \text{cell}^{-1}$. The maximum in Chl *a* concentrations was observed at an I_g of $50 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. In contrast to the response of PE and PC, Chl *a* continued to decrease at irradiances $>700 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and up to 50% of the change in Chl *a* concentration was expressed at irradiances $>I_{\text{sat}}$.

Cells grown at 30, 160 and $1330 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ showed pronounced differences in the extent of thylakoid surface area when viewed in thin sections by electron microscopy (Fig. 5). Cells grown at $1330 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ had a single thylakoid located 40–60 nm to the inside of the cell membrane. At $160 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, cells exhibited the outer thylakoid band plus a partial band 40–60 nm inside the outer thylakoid. At the lowest experimental irradiance, $30 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, from 2 to 3 thylakoids were observed, with most cells exhibiting a partial third band.

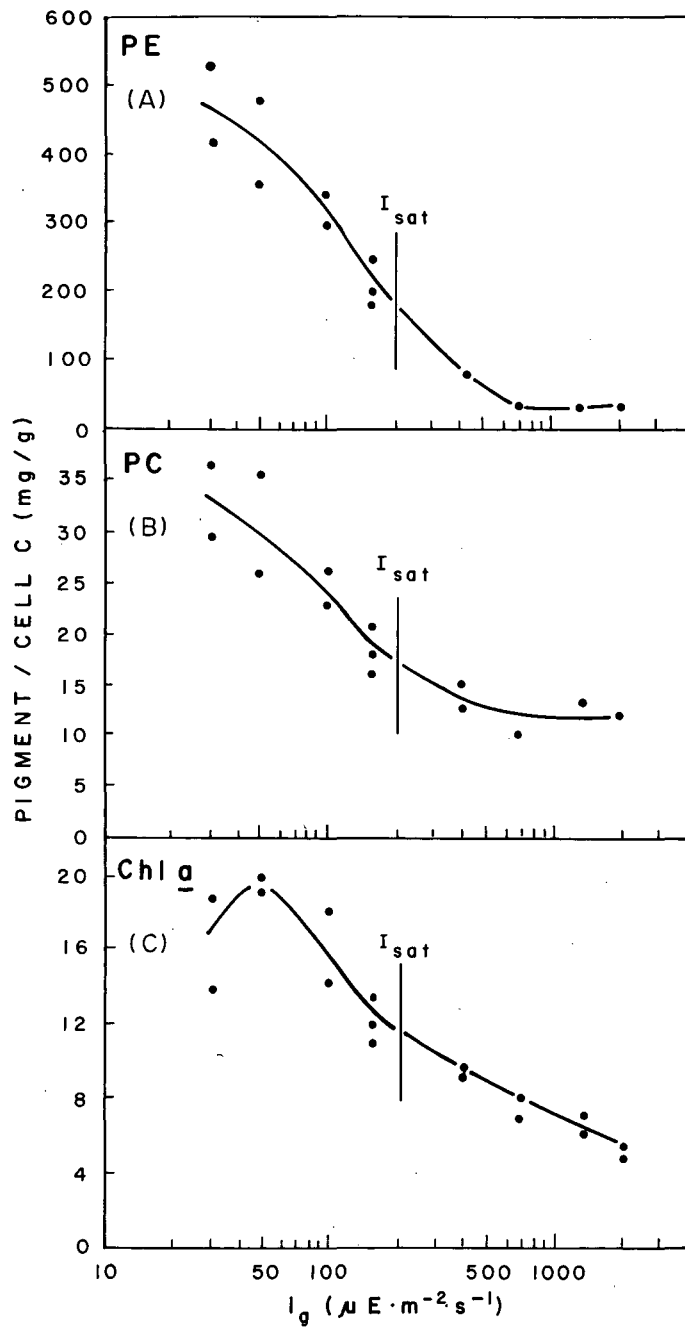


Fig. 4. The effect of irradiance on photosynthetic pigment concentrations of *Synechococcus* WH7803: Concentrations are expressed per cell C (note that concentrations per cell are given in text). I_{sat} is the growth saturating irradiance. (A) PE concentration. (B) PC concentration. (C) Chl *a* concentration.

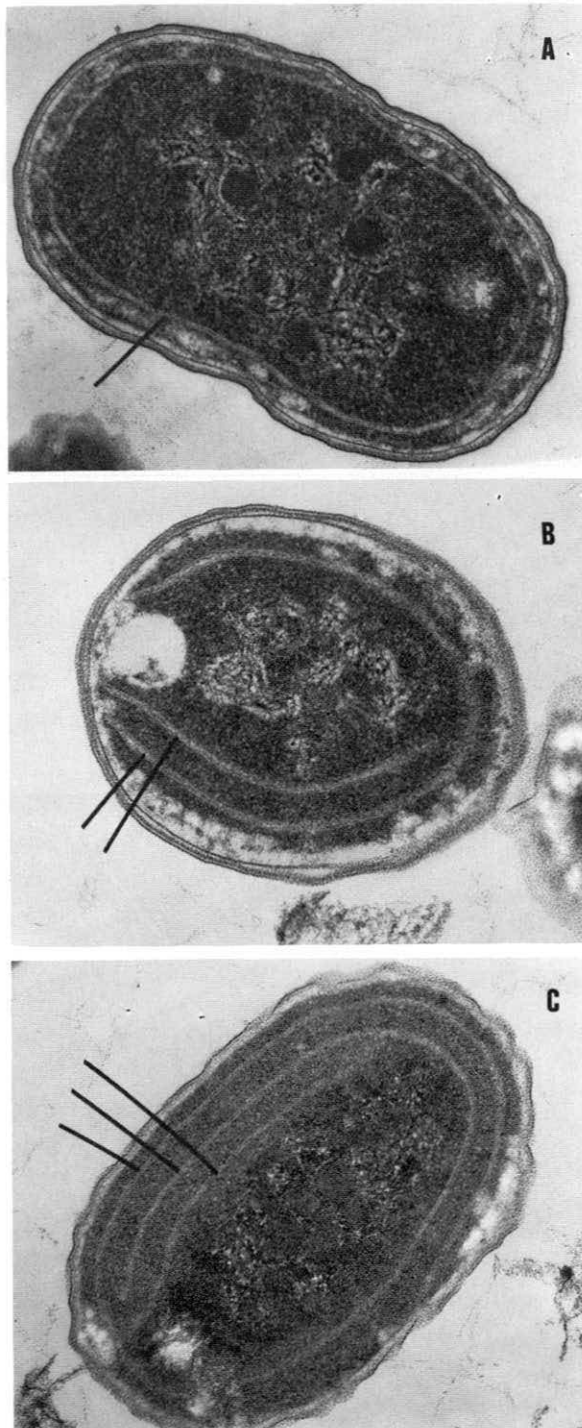


Fig. 5. Electron micrographs of *Synechococcus* WH7803 grown at three irradiances: $1330 \mu\text{E m}^{-2} \text{s}^{-1}$ (A), $160 \mu\text{E m}^{-2} \text{s}^{-1}$ (B), and $30 \mu\text{E m}^{-2} \text{s}^{-1}$ (C). Arrows indicate inward stacking of the thylakoids.

DISCUSSION

Growth

The ecological relevance of irradiances in excess of $200 \mu\text{E m}^{-2} \text{s}^{-1}$, or I_{sat} , to marine *Synechococcus* is an important issue because significant changes in cellular pigment concentrations, and hence spectral quality, were observed at irradiances which saturated growth. We take as a frame of reference a surface mixed layer of clear oceanic water typical of where *Synechococcus* WH7803 was isolated (ALBERTE *et al.*, 1984). If we assume incident radiation of $2000 \mu\text{E m}^{-2} \text{s}^{-1}$, the irradiance at the mid-point depth (*ca.* 20–30 m) would be *ca.* $600 \mu\text{E m}^{-2} \text{s}^{-1}$, assuming an attenuation coefficient of 0.04 m^{-1} . Inclusion of upwelled and scattered light would raise this estimate, and clearly, short-term exposure of cells to even higher irradiances would occur near the surface. Results from this study indicate that the irradiance level in near-surface waters is not necessarily inhibitory to *Synechococcus* and may, in fact, promote high growth rates. We have also found that these high irradiances support maximal growth rates of the clone *Synechococcus* WH8018 (KANA, unpublished data), isolated from a coastal environment (near Woods Hole, Massachusetts; ALBERTE *et al.*, 1984), suggesting that high light competence may be a general characteristic of marine *Synechococcus*.

The capacity for high growth rates of *Synechococcus* WH7803 at irradiances as high as $2000 \mu\text{E m}^{-2} \text{s}^{-1}$, however, depended on preconditioning low-light grown cells step-wise through several intermediate irradiances. Inhibition of growth and photosynthesis under moderate to high irradiance was only observed when low light adapted cells were subjected to large and sudden increases in irradiance (KANA and GLIBERT, 1987; unpublished data). We believe this was also the reason BARLOW and ALBERTE (1985) observed no growth of *Synechococcus* at $250 \mu\text{E m}^{-2} \text{s}^{-1}$ in the laboratory, whereas we observed near-maximal growth rates: they indicated that their experimental cultures were not provided with a period of acclimation to the experimental irradiances prior to measuring growth rates. The capacity of cells to tolerate shifts-up in irradiance has not been determined by experiment so the maximum rate at which cells can adjust is not known. But our recent experience is that the cells can adapt at an even faster rate than that elicited by the protocol outlined above. It is apparent, however, that the photoresponse depends on the timing and magnitude of changes in irradiance and that short-term measurements of growth and photosynthesis rates depend on the extent to which a culture has reached steady-state growth. We are confident that the irradiance response of *Synechococcus* WH7803, as described here, closely reflects the response of cells growing in steady-state, because cultures were maintained for >2 weeks in exponential phase in saturating nutrient concentrations and at cell densities which caused little or no self-shading.

Cellular organization of pigments

In view of recent indications that marine *Synechococcus* behaves in a novel manner with respect to PE function (KURSAR *et al.*, 1981; BARLOW and ALBERTE, 1985; WYMAN *et al.*, 1985), we present an analysis of our data in terms of the structural organization of the photosynthetic pigments based on the known molecular architecture of the light harvesting machinery in cyanobacteria. The modular construction of the phycobilisome (see reviews by GLAZER, 1981; TANDEAU DE MARSAC, 1983; COHEN-BAZIRE and BRYANT, 1982) and almost complete separation of chlorophyll from photosystem II (JONES and MYERS,

1964; MIMURO and FUJITA, 1977; MANODORI and MELIS, 1984) provide the bases for an analysis of pigment ratios. We have shown that PE exhibited a greater plasticity than PC (Figs 4A, B). This caused the PE:PC (w/w) ratio to change with I_g in a non-linear but well-defined manner (Fig. 6). Over the range 30–100 $\mu\text{E m}^{-2} \text{s}^{-1}$, the ratio decreased from 14 to 13, but from 100 to 700 $\mu\text{E m}^{-2} \text{s}^{-1}$ the ratio decreased linearly with the log of irradiance to a value of *ca.* 2.5. Much of this decrease occurred at irradiances $>I_{\text{sat}}$. At irradiances between 700 and 2000 $\mu\text{E m}^{-2} \text{s}^{-1}$, the ratio remained at the minimum value. These changes are consistent with the known molecular architecture of cyanobacterial phycobilisomes where the physical arrangement of phycobiliproteins (e.g. PE and PC) corresponds to the path of energy flow to the photosynthetic reaction centers (primarily photosystem II) situated on the thylakoid membranes (see reviews by GLAZER, 1981; HO and KROGMANN, 1982). In PE-containing organisms, short wavelength (550 nm) absorbing PE discs occupy the ultimate position and longer wavelength (648 nm) absorbing PC discs the penultimate position of the rod structures, thus effecting a directional transfer of energy to the phycobilisome core (GLAZER *et al.*, 1985a,b).

At high irradiances ($I_g > 700 \mu\text{E m}^{-2} \text{s}^{-1}$), the constant ratio and minimum concentrations of PE and PC indicated that (a) PE was always synthesized in the cell and (b) there was a minimum phycobilisome size reached at *ca.* 700 $\mu\text{E m}^{-2} \text{s}^{-1}$. That these pigments were also functionally attached to the photosynthetic membrane, and were not primarily unassembled precursors to phycobilisomes, was indicated by excitation spectra for chlorophyll fluorescence measured at 680 nm (T. KANA, unpublished data). Major excitation peaks for Chl *a* fluorescence were observed at 550 and 648 nm, corresponding to the absorption peaks of PE and PC, respectively (WOOD *et al.*, 1985), thus indicating the transfer of energy from these phycobiliproteins to Chl *a*. Moreover, the ratio of 550:648 nm induced chlorophyll fluorescence was correlated with the PE:PC ratio (T. KANA, unpublished data) indicating that the observed changes in phycobilisome-coupled pigments were correlated with the observed changes in pigment concentration.

The increase in the PE:PC ratio as I_g decreased from 700 to 100 $\mu\text{E m}^{-2} \text{s}^{-1}$ is

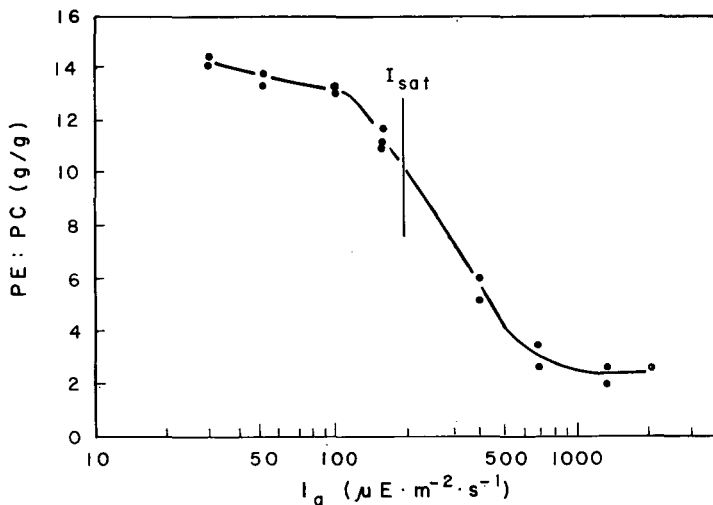


Fig. 6. The effect of growth irradiance on the ratio of PE:PC (w:w) of *Synechococcus* WH7803. I_{sat} is the growth saturating irradiance.

consistent with an outward stacking of additional PE discs on the phycobilisome rods (GLAZER, 1981). The effect would be to physically increase the size of the individual phycobilisome in low light grown cells and increase the absorption cross-section of the photosynthetic unit (PSU).

At $I_g < 100 \mu\text{E m}^{-2} \text{s}^{-1}$, the increase in the PE:PC ratio was small (13–14), but increases in the PE and PC concentrations (Figs 4A, B) were substantial (ca. 40%), indicating that while individual phycobilisomes were near the maximum size, the number of phycobilisomes per cell increased significantly. It is also likely that the PC concentration is correlated with the number of phycobilisomes assuming that the number of rods per phycobilisome is conservative (this later point is unknown for *Synechococcus* WH7803). Since PE was always present and coupled to the phycobilisome, even at the highest irradiances (see above), it is unlikely that there was variation in the number of PC discs situated between PE and the core components in this organism. It can be seen that the most significant increase in PC per cell occurred at irradiances $< I_{\text{sat}}$, suggesting that phycobilisome number changes primarily in response to irradiances which limit growth rate. This is the same general conclusion that was drawn by interpreting the PE:PC ratio and PE and PC concentration data. Thus, *Synechococcus* WH7803 responds to irradiance by adjustments in both phycobilisome number and size, and these different means of adaptation occur over different portions of the light intensity gradient. Changes in phycobilisome size occur primarily between 700 and $100 \mu\text{E m}^{-2} \text{s}^{-1}$, or over irradiances which saturate or nearly saturate growth rate (Fig. 6), whereas changes in phycobilisome number occur primarily over irradiances which limit growth rate. These changes are also discussed in the following paper (KANA and GLIBERT, 1987) in the context of photosynthetic behavior.

The observed changes in pigment concentrations were correlated with changes in thylakoid area (cf. ALLEN, 1968; WAALAND *et al.*, 1974, RAPS *et al.*, 1985). Our observations of the increase in thylakoid surface area at low light are strikingly similar to the differences observed by JOHNSON and SIEBURTH (1979) in samples collected from different depths. They showed that cyanobacteria from 25 m in a North Atlantic site had only a single thylakoid band (their Fig. 1A); this resembled cells grown at $1330 \mu\text{E m}^{-2} \text{s}^{-1}$ in our study (Fig. 5). It is likely that the depth from which that particular sample was taken was within the surface mixed layer. A sample from 25 m on Georges Bank (also probably from the surface mixed layer) contained ca. 1.5 bands (their Figs 2A and B); this resembled cells grown at $160 \mu\text{E m}^{-2} \text{s}^{-1}$ in our study. This latter site is generally more turbid than the former and cells were most likely exposed to a somewhat lower average irradiance. By contrast, cells collected from 80 to 100 m at a variety of oceanic locations had 2–4 thylakoid bands (their Figs 2C–H); this most closely resembled cells grown at $30 \mu\text{E m}^{-2} \text{s}^{-1}$ in our study. It seems apparent that the cells from 80 to 100 m were trapped at or below the pycnocline and were low light adapted. Given the observed differences in thylakoid morphology in surface and deep samples and the correlation with what we have found in the laboratory, it is likely that photoadaptation occurs in field populations, a conclusion also arrived at by GLOVER *et al.* (1985) based on measurements of photosynthesis.

Nitrogen storage considerations

A demonstrated response of cyanobacteria is the mobilization of phycobilisome-bound phycobiliproteins during periods of nitrogen deprivation (YAMANAKA and GLAZER,

1980). This can be considered an adaptive response because the mobilization is of nitrogen-rich compounds which, although supportive of photosynthesis, are not essential to the continuation of the photosynthetic process (LEMASSON *et al.*, 1973). The ability of the cell to buffer periods of nitrogen deprivation therefore would be a function of the concentration of phycobiliprotein in the cell.

Ignoring momentarily the issue of whether *Synechococcus* WH7803 has a non-phycobilisome bound or "free" PE pool, we may simply ask how much nitrogen is available in total cellular PE in rapidly growing cells. We have seen that there is a >20-fold change in PE concentration depending on I_g . Estimates of the amount of cell nitrogen contained in PE (assuming 15% of the weight of PE is N) are shown in Fig. 7. Low light grown cells had >20% of their nitrogen in PE, whereas high light grown cells contained <3% of the total cell nitrogen in PE. It is clear that rapidly growing, nutrient replete cells sequester little nitrogen in PE or other phycobiliproteins.

Moreover, high light grown cells do not divert nitrogen from PE to some other nitrogen-containing compound as demonstrated by the following calculation. The amount of nitrogen required to reduce the C:N (moles) ratio from 6.3 to 4.5 (assuming a constant 250 fg C cell⁻¹) was 18.5 fg cell⁻¹. This would be the amount of nitrogen in *ca.* 125 fg protein and this is only slightly more than the increase in PE + PC observed in these cells over the same irradiance range. The increase in the phycobiliprotein concentration almost totally accounted for the observed decrease in the C:N ratio as cells adapted to lower irradiances, indicating that the total concentration of non-phycobiliprotein nitrogen-containing compounds was relatively insensitive to irradiance levels. Stated differently, adaptation to growth-limiting irradiances caused changes in phycobiliprotein concentration and this was reflected in the C:N ratio. We address the photoadaptive nature of the phycobiliprotein changes in the following paper (KANA and GLIBERT, 1987).

The low concentration of phycobiliprotein in cells growing at high but ecologically relevant irradiances requires us to refine our notions of the role of phycobiliprotein in supporting growth during periods of nitrogen insufficiency. High light grown cells have *ca.* 15–20% less nitrogen than low light grown cells. There are likely to be at least two

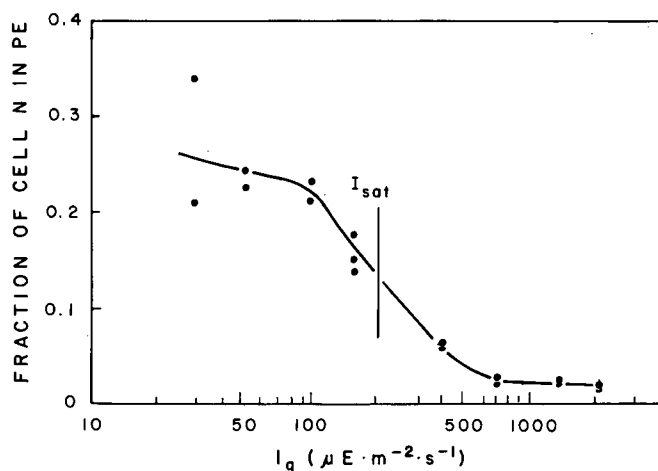


Fig. 7. The effect of growth irradiance on the cellular partitioning of nitrogen in PE in *Synechococcus* WH7803.

important consequences of reduced nitrogen content due to reduced phycobiliprotein content. First, the interval during which growth and maintenance occurs at the expense of cell constituents (e.g. during periods of nitrogen starvation) would be shorter for high light adapted cells than for low light adapted cells. The importance of this difference in terms of population growth would be most pronounced in environments exhibiting relatively short intervals of nitrogen starvation, since long-term survivorship would likely be more dependent on the ability of the cell to alter the rate and pathways of metabolism. Short-term, rather than long-term starvation conditions, however, are likely to be characteristic of the oceanic environment. Second, the material (e.g. amino acids) that can be supplied by catabolism of phycobiliprotein during nitrogen starvation is considerably less in high light adapted cells. After exhaustion of the (small) phycobiliprotein pool, further breakdown of cell constituents would likely involve components which are more critical to maintaining the viability of the cell.

With respect to the notion that *Synechococcus* WH7803 contains a non-phycobilisome bound pool of PE, the behavior of *Synechococcus* that we observed in this and a previous study (GLIBERT *et al.*, 1986) is inconsistent in two ways. First, the ratio of PE:PC at low growth rates, where cellular concentrations of PE were highest, was relatively invariant. If a significant free pool of PE did exist, we would expect that this ratio would continue to increase as concentrations of PE increase. Moreover, we have also found that cultures of *Synechococcus* WH7803 when grown at $80 \mu\text{E m}^{-2} \text{s}^{-1}$ (where PE is not minimized) did not continue to grow, even at a reduced rate, when nitrogen deficient for a period of at least 4 days (GLIBERT *et al.*, 1986). Flow cytometry measurements on these same cultures indicated that during the period of nitrogen deprivation, PE fluorescence remained high, chlorophyll (red) fluorescence declined and the cells increased in size. These results suggested that little to no mobilization of PE (free or phycobilisome-bound) occurred to support growth. In contrast, the coastal isolate of *Synechococcus*, WH8018, continued to divide during the period of nitrogen deprivation, and both PE and chlorophyll fluorescence declined precipitously (GLIBERT *et al.*, 1986). Thus, there appear to be strain-specific differences in the regulation of growth of *Synechococcus* under nitrogen-deficient conditions, which likely reflect different adaptations to the different rates of nitrogen supply in the water columns in which they normally grow (GLIBERT *et al.*, 1986). These findings are not in agreement with those of WYMAN *et al.* (1985) for clone WH7803, and we have previously addressed some of the reasons why we think this is so (GLIBERT *et al.*, 1986).

It is apparent that *Synechococcus* can overcome any potential photoinhibitory effects of surface irradiances when suitably conditioned. Based on our results, we suggest that *Synechococcus* can grow at maximal rates in surface mixed layer environments that are not nutrient limited, and that these high growth rates can be accomplished, and are indeed correlated, with low concentrations of photosynthetic pigments. The low cell PE fluorescence that is often observed in surface populations of *Synechococcus* may not necessarily be an indication of stress, but rather may be a consequence of a photoadaptive reduction in photosynthetic pigmentation in response to saturating irradiances. We observed discernable, but little, fluorescence using standard epifluorescence microscopy techniques in high light grown cells compared to low light grown cells. Yet, these cells were growing at maximal growth rates.

In conclusion, we have found that (a) high irradiance levels alone are not a barrier to rapid growth of *Synechococcus* in marine surface waters, a contradiction of previous

experimental evidence, (b) the pigmentation of WH7803 changes over both limiting and saturating irradiance, but in different ways, and (c) PE concentrations in high light grown cells are so low as to be a relatively ineffective nitrogen reserve. In the following paper we address the photosynthetic response and its relationship to pigment concentrations.

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