Oecologia (Berlin) (1984) 61:143–149

Oecologia

Original papers

Cyanobacterial carotenoids: their roles in maintaining optimal photosynthetic production among aquatic bloom forming genera

Hans W. Paerl
Institute of Marine Sciences, University of North Carolina, Morehead City, NC 28557, USA

Summary. Photoprotective and photosynthetic roles of carotenoid pigments (xanthophylls and β-carotene) were examined in the major bloom forming blue-green algal (cyanobacterial) genera, *Anabaena*, *Aphanizomenon* and *Microcystis*. Since these genera often reside as scums in surface waters, attention was given to the ability of carotenoids to counter potential photooxidation due to maximum near U.V. and visible radiation as well as O_2 supersaturation, characterizing surface waters supporting blooms. In U.V.-transparent quartz incubation flasks it was shown that inhibition of carotenoid synthesis by diphenylamine led to rapid photooxidation among the above genera. When carotenoid synthesis was allowed to proceed, a high degree of resistance to photooxidation resulted. Prolonged exposure to near U.V. irradiation led to enhanced carotenoid synthesis relative to chlorophyll *a*, which extended viability. Carotenoid enhancement also increased chlorophyll *a*-specific photosynthetic O_2 production. It is concluded that enhanced carotenoid synthesis observed during blooms serves at least two ecological functions, i) providing photoprotection and ii) increasing photosynthetic performance of surface cyanobacterial populations.

Introduction

A unique characteristic among the major bloom-forming blue-green algal (cyanobacterial) genera, *Anabaena*, *Aphanizomenon* and *Microcystis*, is the appearance, and at times proliferation, of surface populations. These populations accumulate as scums on calm days, when vertical mixing and/or water currents fail to offset cellular buoyancy. Buoyancy is attributable to intra-cellular gas vacuolation, the regulation of which has been examined in detail by Walsby and coworkers (Walsby 1972; Dinsdale and Walsby 1972; Walsby and Booker 1980). Diverse studies (Reynolds and Walsby 1975; Booker and Walsby 1981; Paerl and Ustach 1982) have shown that vacuolation is related to photosynthetic performance, in that concentrations of cellular photosynthate are inversely related to degrees of gas vacuolation. It follows that if optimal photosynthetic rates are restricted, either due to photosynthetically active radiation (PAR, 400–700 nm), CO_2 or nutrient constraints, the resultant low cellular photosynthate levels lead to increased buoyancy. Residence near the air-water interface offers an attractive solution to either light or CO_2 limitation of photosynthesis, particularly in highly colored eutrophic waters often inhabited by these bloom genera. Buoyancy, and resultant surface accumulations, may therefore be an adaptive response to the above photosynthetic constraints.

However, extensive residence in surface waters has been shown to lead to photooxidation, a potentially lethal condition among a variety of cyanobacteria (Abeliovich and Shilo 1972; Eloff et al. 1976). Photooxidative damage has been attributed to contemporaneous O_2 supersaturation and high irradiance levels in surface waters. It appears that residence in surface waters alleviates PAR and CO_2 limitation, but exposes cells to potential photooxidation. How then do cyanobacteria maintain optimal growth conditions, given this paradoxical situation?

It would be essential that canobacterial surface populations possess mechanisms allowing for a balance between optimal photosynthesis and potential photoinhibition leading to photooxidation. It is important that we recognize and understand such mechanisms, for they may enable surface populations of the above nuisance species to persist and proliferate.

Several field and laboratory studies have implicated cellular carotenoids (xanthophylls and β-carotene) as being important accessory pigments instrumental in absorbing low-wavelength PAR (400–550 mm) as well as near U.V. irradiation (360–400 mm) (Buckley and Houghton 1976; Paerl et al. 1983). As accessory pigments, carotenoids have the ability to transfer captured radiant energy to chlorophyll *a*, thereby increasing photosynthetic production potentials (Clayton 1982). As absorbers of near U.V. irradiation, carotenoids are considered to be effective in protecting a variety of biologically active compounds, including chlorophyll *a*, from photooxidation (Goodwin 1981). Results presented here are in part a confirmation of the dual role that carotenoids play by: i) enhancing cellular photosynthetic production, and ii) providing photooxidative protection in bloom-forming cyanobacteria. The ability of these cyanobacteria to regulate cellular carotenoid content in response to qualities, magnitudes and duration of ambient near U.V. irradiation has also been investigated. The relation of cellular carotenoid concentrations to chlorophyll *a*-specific photosynthetic O_2 evolution rates was examined in order to observe potential links between photosynthetic performance and carotenoid content in natural cyanobacterial populations.

Methods and materials

Incubation and experimental conditions

The following cyanobacteria were obtained from nature and purified in the laboratory: 1) *Anabaena oscillarioides*, originally obtained in 1976 by Dr. K. Lam from a bloom in the Waikato River, New Zealand. This organism was grown axenically in batch cultures, using Chu-10 (nitrogen free) medium (Chu 1942). Cultures were grown at 27° C in 9 l Pyrex bottles fitted with a teflon-coated magnetic stirrer and filtered air supply. Illumination was provided by a mixture of gro-lux and cool-white fluorescence at 400 μ Einsteins·m^{-2}·s^{-1}. *Microcystis aeruginosa* was obtained in 1982 during surface blooms in the Neuse River, North Carolina (Paerl et al. 1983). This colonial cyanobacterium grew well on ASM-J medium (Parker 1982) in 2 liter non-axenic batch cultures having magnetic stirrers. Incubation temperature was 28° C and illumination was 600 μEinsteins·m^{-2}·s^{-1} gro-lux/cool-white fluorescence. *Aphanizomenon flos-aquae* was isolated during 1982 from the Chowan River, North Carolina by the author under non-axenic conditions. Batch cultures of 1 l were grown at 28° C using Chu-10 (minus nitrogen) as media. Gro-lux/cool-white illumination of 400 μEinsteins·m^{-2}·s^{-1} was provided. This highly buoyant species grew best under static (non-stirred) culture conditions. Cultures were swirled by hand for 30 s once a day.

For experimental purposes triplicate (for each treatment) 75 ml culture samples were taken and dispensed in sterile 125 ml Erlenmeyer flasks. Flasks were then exposed to various illumination schemes including: i) gro-lux fluorescence (400 μEinsteins·m^{-2}·s^{-1} PAR), ii) cool-white plus gro-lux fluorescence (400 μEinsteins·m^{-2}·s^{-1} PAR), gro-lux + black light blue U.V. and near U.V. illumination (350 μEinsteins·m^{-2}·s^{-1} PAR). All fluorescent lamps were obtained from Sylvania (Genl. Tel. and Electric Co.). Spectral qualities of each lamp type are given in Figure 1. Flasks were all placed on a slowly oscillating orbital shaker in a temperature controlled (28° C) incubator. Incubation flasks were made of both T08 commercial grade quartz (Amersil-Hereaus Corp.) and Pyrex glass.

For examining recent synthesis of photosynthetic pigments $^{14}CO_2$ incorporation was monitored. A 5.5 μCi aliquot (1 ml) of ^{14}C-$NaHCO_3$ solution, 58 μCi·μmole^{-1} specific activity (Amersham Corp.), was dispensed per 75 ml of culture. Following isotope additions, 25 ml subsamples were periodically withdrawn and centrifuged in the dark at 2,600 RPM for 5 min to concentrate cyanobacterial cells. The pellet obtained was then washed with unlabeled media, recentrifuged and sonicated in 90% acetone. HPLC and TLC analysis were conducted on the extracts in order to separate specific carotenoid and chlorophyll fractions and to determine specific radioactivities of those fractions (see HPLC and TLC methodology).

Diphenylamine (DPA), at 7×10^{-5} M, has been successfully used to inhibit carotenoid synthesis in the photosynthetic bacteria *Rhodopseudomonas rubrum* (Goodwin and Osman 1953), *R. spheroides* (Cohen-Bazire and Stanier 1958), and *Chromatium* sp. (Fuller and Anderson 1958). In current studies DPA additions were made to *Microcystis aeruginosa* and *Anabaena oscillarioides* cultures. Preliminary experiments revealed that 3×10^{-5} M DPA was a useful concentration for these cyanobacteria, because effective inhibition of carotenoid synthesis could be accomplished without detrimental effects on either chlorophyll *a* synthesis or cell viability. Stock solutions (3×10^{-2} M) were made up by dissolving purified crystalline DPA (Sigma Chemical Co.) in 70% basic (pH 9.0) ethanol. For experiments a 1:1000 dilution of stock solution was employed.

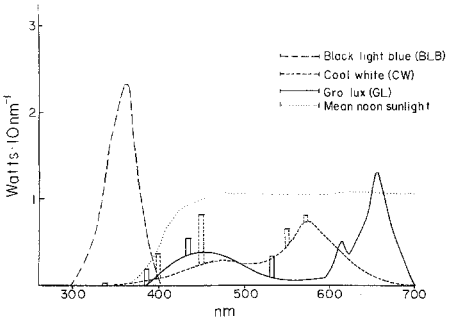

Fig. 1. Relative spectral energy output of various fluorescent illumination sources used in this study. Note that both cool-white and gro-lux reveal distinct peaks as well as emission gradients

The effects of DPA on carotenoid synthesis were monitored by high performance liquid chromatography (HPLC), and spectral absorption scans. Oxygen evolution assays were conducted in order to evaluate photosynthetic performance under the diverse light regimes described above. All DPA treatments were compared to untreated (control) samples incubated under identical illumination.

Photosynthetic performance measurements

Photosynthetic O_2 evolution measurements were made using a Clark-type O_2 electrode (YSI 5750), inserted through a silicone stopper into a 125 ml TO8 quartz Erlenmeyer flask equipped with a magnetic stirring bar. A YSI 54 ARC oxygen meter connected to a Houston Instruments Omniscribe recorder were used to monitor O_2 evolution. This system proved sensitive enough to record O_2 evolution within a 5 min period (Paerl and Ustach 1982), thereby avoiding potential CO_2 and nutrient limitation in sealed incubation flasks during measurements. The illumination regimes described earlier were used. Chlorophyll *a* was extracted from all samples in 90% $MgCO_3$ buffered acetone and quantified according to Burnison (1980). PAR was measured during incubation and O_2 evolution determinations with a Li-Cor 192S quantum PAR sensor, coupled to an LI-185 quantum radiometer.

Pigment analyses

Carotenoid and chlorophyll pigments were examined by high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and scanning spectrophotometry. All samples were initially concentrated either by centrifugation or filtration on 25 mm diameter Whatman GFC glass fiber filters. In both cases small amounts of a saturated $MgCO_3$ solution were added prior to concentration steps in order to assure neutralized conditions prior to pigment extraction. Pigments were extracted by sonicating algae in 90% (buffered pH 8.5) acetone, followed by a 1 h extraction in darkness at −20° C. Extracts were then centrifuged for 10 min at 2600 RPM, followed by filtration of the supernatant through 25 mm diameter 0.2 μm porosity Nuclepore filters. The filtrate was then analyzed by the following tech-

niques. HPLC methodology: A 20 µl filtrate sample was injected into an Altex model 110 A liquid chromatograph, programmed for linear solvent gradient elution. A 25 cm long Ultrasphere ODS-18 reverse phase column was used for separations, and a Hitachi 100-10 spectrophotometer having an 8 µl micro-flow cell served as the detector. Over time, the linear solvent gradient was: 0–7 min = 90% methanol 10% acetonitrile (solvent A), 7–11 min = a linear increase to 60% acetone (the remaining 40% of the mobile phase being solvent A), 11–20 min = 60% acetone (40% solvent A), 20–28 min = a linear decrease in acetone until solvent A was the sole mobile phase. A 1.5 ml·min^{-1} flow rate was used.

Carotenoid and chlorophyll pigments were identified by comparing retention times as well as absorbance characteristics to known carotenoid and chlorophyll *a* reference standards. Reference standards included: i) purified chlorophyll *a* (derived from *Anacystis nidulans*) obtained from Sigma Chemical Company, ii) purified *β*-carotene, also obtained from Sigma Chemical Company, iii) zeaxanthin, obtained from both *Phormidium persinicum* donated by Dr. D. Chapman, freeze dried corn *Zea mais*, and synthesized by Hoffman La Roche, Switzerland, iv) myxoxanthophyll, previously identified and obtained from both *Anabaena oscillarioides* and *Microcystis aeruginosa* (Paerl et al. 1983), v) purified echinenone, obtained from Hoffman La Roche, Switzerland. Prior to the use of standards, respective R_f values were obtained by the use of TLC separations (Paerl et al. 1983). These values were subsequently compared to R_f values for the same pigments determined by other workers (Stransky and Hager 1970; Jeffrey 1974, 1981).

Several wavelengths were employed for specifically detecting either chlorophyll *a*, carotenoids or both groups of pigments. At 663 nm chlorophyll *a* was specifically detected. Maximum absorption peaks for carotenoids were close to 475 nm; accordingly this wavelength was chosen for specifically detecting carotenoids. Together, chlorophyll *a* and carotenoids were effectively detected at 440 nm. On several occasions eluant was collected using a Pharmacia Frac-100 programmable fraction collector, capable of handling 7 ml mini-scintillation vials. A 0.75 ml fraction was collected per vial every 30 seconds.

TLC methodology: Two-dimensional TLC was used for both pigment purification and identification purposes. Avicel micro-crystalline cellulose coated (250 mm thick) 20 × 20 cm glass plates were employed (Analtech, Inc.). The method of Jeffrey (1981) was employed with a minor modification. First-dimensional separation was in n-propanol:ligroine (2.5:97.5 v/v) followed by a second dimensional separation in ligroine:chloroform:acetone (70:30:0.5 v/v/v). The only deviation from Jeffrey's technique was in the use of ligroine instead of light petroleum, which was unavailable. All TLC and HPLC reagents were Fisher HPLC or A.R. grade. Pigments (from filtrate) were initially concentrated by extraction and separation in a petroleum ether and 10% NaCl solution (equal volumes of each), at −20° C for 20 min. The ether phase yielded to pigments; it was collected and concentrated to 30 µl by volatilizing the ether under a stream of helium. Any water remaining after this concentration step was further separated by centrifugation.

Concentrated pigments were applied 2 cm from a corner of each plate and the solvent part was allowed to move 15 cm up the plates. Plates were removed and dried between first- and second-dimensional separations. Clearly-separated spots were measured for R_f values and removed by carefully scraping them off plates, followed by preservation at −20° C in 90% acetone. Pigments purified in this manner were processed further, either for identification or purification purposes, by HPLC.

Scanning Spectrophotometry: Filtered extracts were directly analyzed for absorption characteristics using a Bausch and Lomb model 2000 U.V.-Vis. double beam scanning spectrophotometer. Scans ranged from 800 to 350 nm; 1 cm width quartz micro curvettes were used as sample holders. All scans were plotted on an x-y recorder.

Results

Pigment alterations in response to specific illumination regimes

Alterations in cellular carotenoid to chlorophyll *a* ratios occurred among all three cyanobacterial genera when populations initially grown for a 48 h period in Pyrex flasks under gro-lux (GL) plus cool-white (CW) were transferred to different illumination regimes. Transfer of cyanobacteria from GL plus CW to GL alone or GL plus CW at variable light intensities yielded identical results, namely, no significant alterations in pigment ratios. However, when GL plus CW-grown cultures were transferred to GL plus black light blue (BLB), changes in pigment ratios became evident. All four major carotenoids identified (myxoxanthophyll, zeaxanthin, echinenone as well as *β*-carotene) increased with respect to chlorophyll *a* after prolonged (48 h or longer) exposure to BLB illumination (Fig. 2). This response was most profound in *Microcystis aeruginosa*, a species often found in surface waters, but all three genera, to varying degrees, revealed similar responses (Fig. 2). Incubation in quartz flasks led to the most profound carotenoid increases (relative to chlorophyll *a*) in response to GL plus BLB illumination during 48 h exposures. This indicated that increased carotenoid synthesis was most profound when U.V. and near U.V. illumination were transmitted by the incubation vessels. Differences in total PAR intensity failed to explain the responses observed, since total PAR fluxes were 400, 400, and 350 µEinstein·m^{-2}·s^{-1} for GL plus CW, GL, and GL + BLB respectively. Changes in cellular chlorophyll

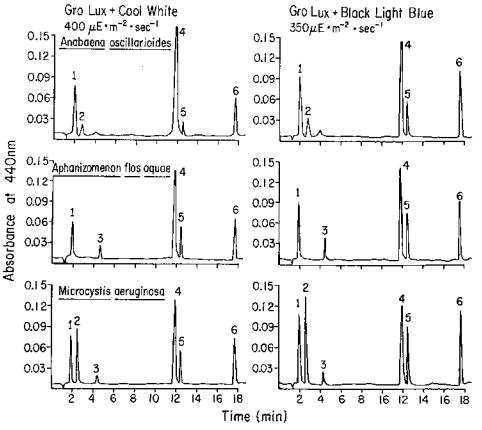

Fig. 2. HPLC separations of carotenoids and chlorophyll *a* in 3 cyanobacterial genera before and 48 h after transfer from gro-lux plus cool white to gro-lux plus black light blue. The following pigment peaks were detected: *1* myxoxanthophyll, *2* zeaxanthin, *3* unidentified carotenoid, *4* chlorophyll *a*, *5* echinenone, and *6* *β*-carotene

Table 1. Changes in *Microcystis aeruginosa* chlorophyll *a* content and carotenoid/chlorophyll *a* ratios in response to gro-lux plus cool white (GL+CW), gro-lux (GL), and gro-lux plus black light blue (GL+BLB) illumination, following a 24 h exposure period in either quartz or pyrex flasks. T_0 represent conditions at the start of the exposure period. Standard error values are given for each set of triplicate measurements. Carotenoid/Chlorophyll *a* ratios were derived by absorbance measurements at 480 and 663 nm respectively. Respective extinction coefficients of 2100 $(mg/ml)^{-1}\cdot cm^{-1}$ and 61 $(mg/ml)^{-1}\cdot cm^{-1}$ were used to estimate concentrations

	Chlorophyll *a* ($mg\cdot l^{-1}$)			Carotenoid/Chlorophyll *a* (mg/mg)		
	T_0	Quartz	Pyrex	T_0	Quartz	Pyrex
	2.05±0.12			0.25±0.08		
GL+CW		3.02±0.15	2.94±0.21		0.41±0.05	0.38±0.06
GL		2.89±0.17	2.67±0.26		0.38±0.04	0.36±0.05
GL+BLB		2.64±0.29	2.81±0.33		0.51±0.09	0.42±0.07

a content, in response to any of the above irradiation treatments, were minor compared to changes in specific carotenoid to chlorophyll *a* ratios (Table 1). Hence, increased carotenoid to chlorophyll *a* ratios in response to the presence of BLB light were due to enhancement of cellular carotenoid levels.

This finding was further investigated by specifically assessing the fate of recently assimilated ^{14}C with respect to pigments under investigation. Cultures, similar to those described above, were grown in the presence of ^{14}C-$NaHCO_3$ for 48 h, followed by 90% acetone extraction for carotenoids and chlorophyll *a*. A highly concentrated extract was examined by TLC. The spots corresponded to the following known two-dimensional R_f values for cyanobacterial carotenoids (Stransky and Hager 1971; Jeffrey 1981; Paerl et al. 1983); myxoxanthophyll=0.082/0.008, zeaxanthin=0.380/0.340, echinenone=0.88/0.49, *β*-carotene=0.962/0.970 and chlorophyll *a*=0.843/0.293. Purified standards were also used to confirm specific carotenoid pigments. TLC spots of specific pigments were cut out, resolubilized in 90% acetone and processed by HPLC. The HPLC eluant fractions were collected and assayed for ^{14}C content. Incubations conducted in quartz flasks were compared to those incubated in Pyrex flasks, using the combination of GL and BLB as a light source. Comparisons of both carotenoid:chlorophyll *a* ratios as well as ^{14}C accumulation in specific pigment fractions illustrate the enhancement of carotenoid synthesis in quartz as opposed to clear Pyrex flasks. Generally, carotenoid pigments showed 30 to 50% more ^{14}C incorporation in quartz as opposed to clear Pyrex incubation flasks (Fig. 3). It is therefore concluded that the higher abundance of U.V. and near U.V irradiation transmitted by quartz flasks led to enhancement of carotenoid synthesis.

Diphenylamine (DPA) additions at 3×10^{-5} M effectively inhibited carotenoid synthesis either in the presence or absence of BLB in all three genera, while chlorophyll *a* synthesis, as monitored by HPLC absorbance, remained uninhibited (Fig. 4). The inhibiting effects of DPA became apparent as early as 12 h following its addition to cultures, regardless of illumination conditions (Table 2). The selectivity of DPA in specifically inhibiting carotenoid synthesis allowed for both the observation of; i) potential photooxidative damage by BLB irradiation in the absence of carotenoid synthesis, and ii) comparative photosynthetic performance of control cultures supporting carotenoid enhancement vs. cultures in which carotenoid enhancement was inhibited by DPA.

The potential for photooxidative damage was greatly

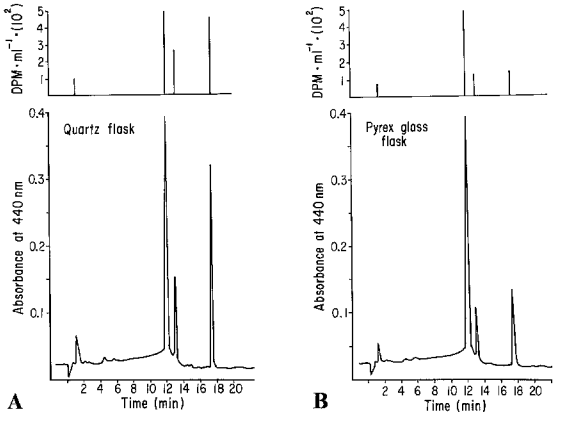

Fig. 3A, B. HPLC separations analyzed for radioactivity (^{14}C) and absorbance at 440 nm. Concentrated *Microcystis aeruginosa* populations grown with $NaH^{14}CO_3$ for 48 h in quartz and Pyrex flasks were analyzed. Illumination was provided by gro-lux plus black light blue fluorescence at 350 μEinsteins$\cdot m^{-2}\cdot s^{-1}$

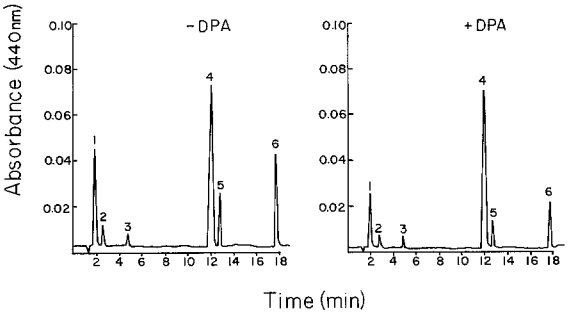

Fig. 4. Effects of diphenylamine (+DPA) on carotenoid and chlorophyll *a* synthesis in *Anabaena oscillarioides* monitored by HPLC. The following peaks were detected: *1* myxoxanthophyll, *2* zeaxanthin, *3* unidentified carotenoid, *4* chlorophyll *a*, *5* echinenone, and *6* *β*-carotene. Cultures were illuminated by gro-lux fluorescence at 400 μEinsteins$\cdot m^{-2}\cdot s^{-1}$ and DPA was added 24 h prior to analyses

enhanced in the presence of DPA during long-term (greater than 12 h) incubations. All genera showed this response, as witnessed by rapid declines in all photosynthetic pigments, particularly cellular chlorophyll *a*, as well as O_2 evolution rates per mg chlorophyll *a* (Table 3). Following a 48 h incubation with 3×10^{-5} M DPA under GL+BLB, *Microcystis aeruginosa* revealed photooxidative damage as opposed to DPA addition under GL illumination alone (Table 3). *Anabaena oscillarioides* revealed slightly higher resis-

Table 2. Influence of diphenylamine (DPA), at 3×10^{-5} M, on chlorophyll *a* concentrations ($mg \cdot l^{-1}$) and carotenoid/chlorophyll *a* ratios (mg/mg) determined spectrophotometrically in 90% acetone extracts from *Anabaena oscillarioides* and *Microcystis aeruginosa*. DPA inhibition of carotenoid synthesis was examined under various illumination regimes. Respective PAR intensities of illumination regimes are identical to those given in Table 1. All samples were incubated in 100 ml quartz containers for 12 h

Illumination	*A. oscillarioides*				*M. aeruginosa*			
	−DPA		+DPA		−DPA		+DPA	
	Chl *a*	Carot/Chl *a*	Chl *a*	Carot/Chl *a*	Chl *a*	Carot/Chl *a*	Chl *a*	Carot/Chl *a*
GL+CW	1.51±0.17	0.26±0.08	1.55±0.21	0.22±0.05	2.41±0.11	0.31±0.08	2.29±0.31	0.25±0.07
GL	1.48±0.19	0.28±0.09	1.61±0.26	0.26±0.07	2.44±0.17	0.32±0.06	2.51±0.23	0.22±0.09
GL+BLB	1.39±0.24	0.45±0.12	1.29±0.19	0.24±0.06	2.26±0.22	0.49±0.08	2.06±0.19	0.26±0.10

Table 3. Effects of long-term exposure of *Anabaena oscillarioides* and *Microcystis aeruginosa* to 3×10^{-5} M diphenylamine (DPA) in combination with U.V.-poor (GL at 400 μEinsteins·m^{-2}·s^{-1}) and U.V.-rich (GL+BLB at 350 μEinsteins·m^{-2}·s^{-1}) illumination. Chlorophyll *a* concentrations (in $mg \cdot l^{-1}$) and O_2 evolution rates (in mg O_2·mg Chl $a^{-1} \cdot h^{-1}$) were monitored as response parameters. T_0 indicate conditions at the start of exposure periods. All incubations were conducted in triplicate 100 ml quartz erlenmeyer flasks. ND indicates no detectable rate

Treatments	*A. oscillarioides*						*M. aeruginosa*					
	T_0		24 h		48 h		T_0		24 h		48 h	
	Chl *a*	O_2 Evol.	Chl *a*	O_2 Evol.	Chl *a*	O_2 Evol.	Chl *a*	O_2 Evol.	Chl *a*	O_2 Evol.	Chl *a*	O_2 Evol.
	1.05	9.23					1.29	6.64				
GL (−DPA)			2.12	9.46	2.56	8.66			2.49	6.44	3.11	6.16
GL (+DPA)			1.85	8.16	2.02	7.19			1.98	5.59	2.22	5.05
GL+BLB (−DPA)			1.60	7.83	1.89	6.92			2.24	6.30	2.65	5.19
GL+BLB (+DPA)			1.21	4.27	1.15	1.55			1.07	2.13	0.89	ND

tance to photooxidation within 48 h; nevertheless, at both 24 and 48 h time intervals the combination of DPA and BLB arrested increases in chlorophyll *a* and severely depressed O_2 evolution rates. Since growth of both species readily continued in the absence of BLB, indications were that DPA was not directly toxic to cyanobacteria within incubation periods examined. Furthermore, total PAR levels were nearly identical either in the presence or absence of BLB, suggesting that light quality rather than quantity promoted photooxidative responses. In a separate set of experiments the PAR intensity was increased to 1200 μEinsteins·m^{-2}·s^{-1}, approximating surface PAR levels. Photooxidative conditions were still not evident in *Microcystis aeruginosa* cultures exposed to these PAR levels, as long as BLB was not a component light source. These results substantiate previous findings that, although cyanobacteria generally exhibit efficient photosynthetic rates (per unit chlorophyll *a*) at low irradiance levels, photoinhibition is uncommon at high (in the range of 800–1200 μEinsteins·m^{-2}·s^{-1}) PAR levels (Kellar and Paerl 1980; Foy and Gibson 1982; Paerl et al. 1983). The introduction of BLB at all PAR levels tested (350–1200 μEinsteins·m^{-2}·s^{-1}) lead to enhancement of photoinhibition. In the presence of DPA such photoinhibition was quickly replaced by photooxidative death, defined here as the point where O_2 evolution could no longer be detected (Table 3).

When *Microcystis* and *Anabaena* populations previously grown under GL and BLB in Pyrex and quartz containers for 24 h were all transferred to quartz containers, significant differences in chlorophyll *a*-specific photosynthetic efficiences resulted. Quartz-adapted populations revealed 20 to 30% higher O_2 evolution rates per unit chlorophyll *a* than Pyrex-adapted populations (Table 4). This result proved consistent for both genera. Magnitudes of chlorophyll *a* specific O_2 evolution rates were compared to carotenoid:chlorophyll *a* ratios in quartz adapted populations under GL+BLB illumination, resulting in generally strong direct relationships (Fig. 5). Adaptation in quartz containers was not related to light intensity, but rather to light quality. The TO8 quartz containers used were virtually

Table 4. Comparative O_2 evolution rates (mg O_2·mg Chl $a^{-1} \cdot h^{-1}$) in quartz and Pyrex adapted *Microcystis aeruginosa* populations. Both sets of treatments were originally derived from a single population. Cultures were allowed to adapt for 24 h, then placed in quartz flasks and exposed to gro-lux plus black light blue (GL+BLB) illumination at 350 μEinsteins·$m^{-2} \cdot h^{-1}$

	Quartz adapted	Pyrex adapted
1	9.12	7.61
2	8.76	6.81
3	9.50	7.25
$\bar{X}$	9.13	7.22

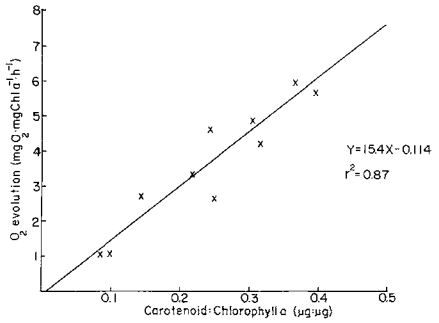

Fig. 5. Relationship between chlorophyll *a*-specific O_2 evolution rates and carotenoid:chlorophyll *a* ratios in *Anabaena oscillarioides* populations. Results from 10 independent incubations under grolux plus black light blue fluorescence (350 μEinsteins·m^{-2}·s^{-1}) are entered in a linear regression analysis

transparent to the near-U.V. light emitted by BLB as well as all GL wavelengths (see Fig. 1 for respective light qualities). Pyrex glass has poor transmittance characteristics in the near U.V. range. Since the visible light spectrum of GL plus BLB is identical to GL alone, differences in photosynthetic efficiencies are likely to reflect more effective near U.V. and low PAR (400–500 nm) light utilization in quartz-adapted populations. Enhancement of cellular carotenoid synthesis would account for more effective light utilization at these wavelengths.

Discussion

The above laboratory results provide support for earlier findings relating specific (per unit chlorophyll *a*) photosynthetic efficiences to carotenoid:chlorophyll *a* ratios observed in natural *Microcystis aeruginosa* populations examined *in situ* in the Neuse River, North Carolina (Paerl et al. 1983). In field studies it was shown that during development of *M. aeruginosa* blooms photosynthetic efficiencies (based on $^{14}CO_2$ fixation measurements) recorded among surface populations increased in parallel with carotenoid (myxoxanthophyll, zeaxanthin and β-carotene) content relative to chlorophyll *a*. From these studies as well as results reported here, it is concluded that increased cellular carotenoid: chlorophyll *a* levels are an adaptive feature of cyanobacterial surface populations, since increased protection from potentially harmful U.V. and near U.V. irradiation as well as increased photosynthetic efficiencies resulted. Hence, the commonly observed yellowing of cyanobacterial surface blooms (Fogg et al. 1972; Kellar and Paerl 1980) more likely reflects an ecological adaptation as opposed to senescent feature of natural populations.

The illumination regimes used here in laboratory experiments do not duplicate natural conditions; the discrete spectral emission regimes of both GL and BLB vary substantially from the more continuous spectral characteristics of incident sunlight at the water's surface (Fig. 1). Nevertheless, it could be shown that BLB irradiation which, like sunlight, is rich in the near U.V. wavelengths (320–400 nm), is instrumental in elevating carotenoid:chlorophyll *a* ratios. Both photoprotective and photosynthetic characteristics of dominant bloom cyanobacteria were enhanced whenever carotenoid enhancement was observed. DPA, which blocks carotenoid synthesis, also adversely affected photosynthetic performance and viability, particularly under BLB illumination.

Research findings presented here on naturally-occuring cyanobacterial bloom species are a confirmation of what have been thought to be the main functional roles of carotenoids in laboratory-grown photosynthetic prokaryotes including *Rhodopseudomonas* (Goodwin and Osman 1953; Cohen-Bazire and Stanier 1958), *Chromatium* (Fuller and Anderson 1958) and *Gloeocapsa* (Buckley and Houghton 1976). Bloom genera such as *Anabaena*, *Aphanizomenon* and *Microcystis* are appropriate organisms for examining the functional roles of carotenoids because they often proliferate in surface waters exposed to maximum U.V., near U.V. and total PAR radiation as well as O_2 supersaturation. Combined, such conditions are known to promote photooxidation (Abeliovich and Shilo 1972; Clayton 1982).

The formation of an excited highly reactive triplet state of chlorophyll *a* is considered to be the initial event in photooxidation. This state is attributable to a combination of high ambient O_2 and light conditions. Once photoxidative conditions arise, carotenoid pigments can provide cellular protection against indiscriminant photooxidation by dissipating energy from highly excited triplet Chl *a* or singlet O_2 states. In this manner carotenoids can play a photoprotective role (Clayton 1982).

Carotenoids are also known to transfer harmless excitation energy in the 400–550 nm region to chlorophyll *a*, thereby increasing the amount of photoreducing energy ultimately transferred to CO_2 fixation by way of chlorophyll *a* (Goodwin 1980). Such transfer can enhance chlorophyll *a*-specific photosynthetic rates as observed in this study.

Both the photoprotective and photosynthetic attributes of carotenoids appear to play roles in observed increased U.V. and near-U.V. tolerances as well as high chlorophyll *a* specific photosynthetic efficiencies reported here for some major cyanobacterial bloom genera. Combined with previously reported field data, evidence exists that carotenoids play a crucial role in cyanobacterial survival, and at times, dominance in potentially photooxidizing surface waters. With specific reference to nuisance blooms, it can be concluded that cellular carotenoid enhancement is a mechanism aiding in the persistance of such blooms, given appropriate nutrient and physical regimes.

Acknowlegements. I would like to thank J. Blackwell, P. Bland and J. Tucker for field and laboratory assistance and D. Chapman, R. Lewin and Hoffman La-Roche, Switzerland for kindly donating carotenoid standards. J. Garner, B. Bright, J. Lewis and V. Page aided with manuscript preparation. This work was supported by the North Carolina Sea Grant Program, The University of North Carolina Water Resources Research Institute and the U.S. National Science Foundation, grant DEB 8104689.

References

Abeliovich A, Shilo M (1972) Photooxidative death in blue-green algae. J Bacteriol 110:682–689

Booker MJ, Walsby AE (1981) Bloom formation and stratification by a a planktonic blue-green alga in an experimental water column. Br Phycol J 16:1–11

Buckley CE, Houghton JR (1976) A study of the effects of near U.V. radiation on the pigmentation of the blue-green alga *Gloeocapsa alpicola*. Arch Microbiol 107:93–97

Burnison BK (1980) Modified DMSO extraction for chlorophyll analysis of phytoplankton. Can J Fish Aquat Sci 37:727–733

Chu SP (1942) The influence of the mineral composition of the medium on the growth of planktonic algae. 1. Methods and culture media. J Ecol 31:109–148

Clayton RK (1980) Photosynthesis: Physical mechanisms and chemical patterns. Cambridge. University Press, London p 281
Cohen-Bazire G, Stanier RY (1958) Inhibition of carotenoid synthesis in photosynthetic bacteria. Nature 181:250–252
Dinsdale MT, Walsby AE (1972) The interrelations of cell turgor pressure, gas vacuolation and buoyancy in a blue-gree alga. J Exp Bot 23:561–570
Eloff JN, Steinitz Y, Shilo M (1976) Photooxidation of cyanobacteria in natural conditions. Appl Environ Microbiol 31:119–126
Fogg GE, Stewart WDP, Fay P, Walsby AE (1973) The blue-algae. Academic Press
Foy RH, Gibson CE (1982) Photosynthetic characteristics of planktonic blue-green algae: The responses of twenty strains grown under high and low light. Br Phycol J 17:169–182
Fuller RC, Anderson IC (1958) Suppression of carotenoid synthesis and its effect on activity of photosynthetic bacterial chromatophores. Nature 181:252–254
Goodwin TW (1980) The biochemistry of the carotenoids. Chapman and Hall, London. p 377
Goodwin TW, Osman HG (1953) Carotenoid synthesis in *Rhodopseudomonas rubrum*. G Biochem J 53:541–545
Jeffrey SW (1974) Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. Mar Biol 26:101–110
Jeffrey SW (1981) An improved thin-layer chromatographic technique for marine pigments. Limnol Oceanogr 26:191–197
Kellar PE, Paerl HW (1980) Physiological adaptations in response to environmental stress during an N_2-fixing *Anabaena* bloom. Appl Environ Microbiol 40:587–595
Paerl HW, Ustach JF (1982) Blue-green algal scums: An explanation for their occurrence during freshwater blooms. Limnol Oceanogr 21:212–217
Paerl HW, Tucker J, Bland PT (1983) Carotenoid enhancement and its role in maintaining blue-green algal (*Microcystis aeruginosa*) surface blooms. Limnol Oceanogr 28:847–857
Parker D (1982) Improved procedures for the cloning and purification of *Microcystis* cultures. J Phycol 18:471–477
Reynolds CS, Walsby AE (1975) Water blooms. Biol Rev 50:437–481
Stransky H, Hager A (1970) Das Carotenoidmuster und die Verbreitung des lichtinduzierten Xanthophyllcyclus in verschiedenen Algenklassen. IV. Cyanophyceae und Rhodophyceae. Arch Mikrobiol 72:84–96
Walsby AE (1972) Structure and function of gas vacuoles. Bact Rev 36:1–32
Walsby AE, Booker MJ (1980) Changes in buoyancy of a planktonic blue-green alga in response to light intensity. Br Phycol J 15:311–319

Received June 22, 1983