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Light quality in relation to growth, photosynthetic rates and carbon metabolism in two species of marine plankton algae

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Abstract

The effect of light quality on growth, photosynthesis and carbon metabolism in two species of marine algae, *Cyclotella nana* (HUSTEDT) and *Dunaliella tertiolecta* (BUTCHER), was examined. Relative growth constants for *C. nana* were 0.37, 0.29 and 0.25 in blue, white and green light, respectively. Corresponding constants were 0.41, 0.31 and 0.29 for *D. tertiolecta*. Photosynthetic rates in both species were higher in blue light and lower in green light compared with white light of the same intensity. More than 60 % of ^{14}C assimilated by *C. nana* or *D. tertiolecta* grown in blue or green light was incorporated into the ethanol-insoluble fraction, compared with 10 to 30 % in this fraction in white light. The relative importance of the various components within this fraction was independent of light quality. Although less ^{14}C was assimilated into the ethanol-soluble fraction in blue or green light, there was a relative increase in some amino acids and organic acids in this fraction and a decrease in sugars and sugar phosphates relative to white light of the same intensity. These differences were independent of light intensity, photosynthetic rate and cell density in the cultures.

Introduction

The chemical composition of higher plants and algae has been determined in a number of species grown under a variety of conditions (for reviews see STRICKLAND, 1960; KROTKOV, 1964). Environmental factors may produce qualitative and quantitative variations in the carbohydrates, amino acids, organic acids and proteins produced from metabolized $^{14}CO_2$. Light intensity (CHAMPIGNY, 1956), temperature (OUELLET, 1951), pH (OUELLET and BENSON, 1952) and factors such as age of cell and stage of life history (NIHEI et al., 1954) have a demonstrated effect on chemical composition. The wavelength of radiation also influences the chemical composition of plants. For example, plants grown under blue light produce more amino acids and protein than those grown under red or white light of the same intensity (VOSKRESENSKAYA, 1956; HAUSCHILD et al., 1962a, b). Further, light quality produces marked differences in growth rates and development (KOWALLIK, 1963; MOHR and HOLL, 1964). Most of this light quality work has been confined to higher plants, although HAUSCHILD et al. (1962a, b), ZAK (1965), HESS and TOLBERT (1967), report that light quality influences the chemical composition of fresh-water algae.

These results suggest that the chemical composition of algae in the photic zone of lakes or oceans may change with depth as a result of differential attenuation of light of the various wavelengths with depth. Thus, blue and green light penetrate to the greatest depths in oceanic and coastal waters, respectively (JERLOV, 1951). Cells at the bottom of the photic zone may be exposed primarily to blue light, which may result in a blue light enhancement of protein synthesis. The nature of the photosynthate may, in turn, influence the quantity and quality of the breakdown products and the dissolved organic compounds algae excrete into the environment. The nutritional value of the algae for herbivores can also be expected to vary with depth.

In this study we examined the effects of blue, green and white light on photosynthesis, growth rates, and the nature of the photosynthate in two marine planktonic algae, *Cyclotella nana* (HUSTEDT) and *Dunaliella tertiolecta* (BUTCHER). This work represents a part of a larger study which will evaluate factors influencing the excretion of dissolved organic compounds by algae.

Material and methods

Axenic cultures of two marine algae, the diatom *Cyclotella nana* (Wood's Hole stock culture Dun) and the green alga *Dunaliella tertiolecta* (Wood's Hole stock culture 3H) were used in these experiments. The cultures were grown in the artificial sea-water medium described by JONES et al. (1963). It was modified by the addition of 1 ml/l vitamin solution containing 500 µg/ml thiamine-HCl, 2 µg/ml vitamin B_{12} and 1 µg/ml biotin. In addition, 10 ml $Na_2SiO_3 \cdot H_2O$ were added to the medium in which diatoms were cultured to yield a concentration of 0.15 g/l. The same volume of 0.01 N HCl as that of silicate was added to maintain a pH of 7.5 to 7.6. The medium was sterilized by autoclaving 20 min at 120 °C and 15 psi.

Growth and photosynthesis measurements

Cultures were grown with continuous stirring at a temperature of 20° ± 0.5 °C in cotton-stoppered 2.8 l "low form" Fernback flasks containing 1.5 l medium. Stock cultures were maintained in white light in screw-capped 125 ml Erlenmeyer flasks containing 50 ml of the medium. Routine checks of both stock and experimental cultures verified the absence of contamination. The inoculum for each experiment was obtained from stock cultures in the exponential phase of growth. The volume of each inoculum was adjusted to provide an initial concentration of 1 to 3×10^4 cells/ml of culture medium.

Growth rates were estimated by measuring the increase in cell numbers with a Model B Coulter Counter, or by measuring changes of optical density at 750 nm with a Spectronic 20 colorimeter. Optical density values were converted to cell numbers by means of a calibration curve. The relative growth constant (k) and mean generation time (T) were calculated from the following formulae:

$$k = \frac{\log_e N - \log_e N_o}{t} = \frac{2.3\,(\log_{10} N - \log_{10} N_o)}{t}$$

and

$$T = \frac{\log_e 2}{k} = \frac{0.693}{k}$$

where N = cell concentration at time t; N_o = cell concentration at time $t = 0$ and t = time in days.

To measure photosynthetic rates, we added 100 µCi of $^{14}CO_3^{2-}$ to each of several replicates of 200 ml of cell suspension taken during the log phase of growth. After 30 min of incubation the experiment was terminated, cell numbers were determined and an aliquot from each suspension was filtered through a Millipore HA filter (47 mm, pore diameter 0.45 µ). The cells were killed and extracted in boiling 80% ethanol for 1 h. The ethanol-insoluble fraction was then removed with a Millipore filter. The filtrate was acidified with 0.1 N HCl to pH 3, aerated vigorously for 1 h to remove $^{14}CO_2$, and then reduced to a small volume *in vacuo* at 28 °C.

Separation and identification of photosynthetic products

Pigments and other compounds soluble in organic solvents were removed from the ethanol-soluble extract by adding a volume of chloroform equal to the volume of the extract. The mixture was shaken and stored for 20 h at 5 °C. After separation, the ethanol-soluble fraction was taken to dryness *in vacuo* at 28 °C. Dried extracts were then redissolved in 1 ml distilled water and a 10 µl aliquot was assayed for ^{14}C activity. The amino acid, organic acid, and sugar fractions were separated on Rexyn 101 (H^+) and Rexyn (OH^-) ion-exchange columns. More than 96% of the total radioactivity present in the ethanol-soluble extracts was recovered in these 3 fractions. A 10 µl aliquot from each fraction was assayed for radioactivity, and 50 µl aliquots were chromatographed on Whatman 3 MM filter paper.

Initially, the amino acids and the neutral preparations were chromatographed in two dimensions with phenol:ammonium hydroxide:water (267:1:37, v/v) and n-propanol:ethyl acetate:water (7:1:2, v/v). After drying, the chromatograms were run a second time in the second solvent system. Glycine, serine, and occasionally glutamic acid were not adequately separated with this two dimensional system. Consequently, the mixture of the amino acids was eluted, spotted on Whatman 3 MM filter paper and developed in a one-dimensional system with borate buffered phenol as described by HAUSCHILD (1959).

Later, we used a second two-dimensional system which gave better separation of these amino acids. The first solvent system consisted of phenol:ammonium hydroxide:water (300:1:75, v/v) and the second solvent system consisted of butanol:acetone:diethylamine:water (11:11:2:5, v/v).

The organic acid chromatograms were developed in two dimensions with ethanol:ammonium hydroxide (95:5, v/v) and the organic phase of butanol:formic acid:water (4:1:5, v/v). This solvent system was prepared 12 h in advance. A clearer separation of organic acids was obtained with a second two-dimensional system consisting of ethanol:water:ammonium hydroxide (35:13:2, v/v) and ethyl acetate:acetic acid:water:sodium acetate (20:11:10:48, v/v).

Chromatograms were exposed to Ilford Ilfex X-ray film for 1 to 3 weeks to locate the radioactive compounds. All radioactive spots were excised and radioactivity in the spots determined. Paper spots were placed directly into 15 ml of a toluene-base scintillator. The efficiency of paper-spot counting was estimated by applying a known activity of ^{14}C glucose to chromatogram paper and measuring the activity under the same conditions as the unknowns. The channels ratio method described by PENG (1966) was also employed for measuring efficiency.

Spots were identified from their relative positions by spray reagents and fingerprinting techniques. Further identification was made by co-chromatography of eluted materials with known compounds.

The ethanol-insoluble residues were converted to $^{14}CO_2$ by the wet combustion method described by HOFSTRA (1967) to measure total ^{14}C fixed in this fraction. In addition, in some experiments, aliquots of the ethanol-insoluble residues were hydrolyzed in a modified Soxhlet apparatus. One portion was hydrolyzed in 1 N H_2SO_4 for 15 h at 105 °C, the other in 6 N HCl for 20 h at 105 °C, for carbohydrate and protein amino acid analysis, respectively. The H_2SO_4 was removed by precipitating with $BaCO_3$, and HCl removed by repeatedly evaporating the hydrolysates to dryness *in vacuo*. The hydrolysates were redissolved

in water and fractionated by ion-exchange resins before the paper chromatographic separation of their constituents.

Light conditions

Newly established algal cultures were placed in controlled environment chambers and exposed to a light:dark regime of 16:8 h. The cells were illuminated from above with white, blue or green fluorescent lamps; in the latter two cases sheets of blue or green celluloid were used as light filters. The energy and wavelength of light at the surface of the cultures was measured with an ISCO Spectroradiometer. The maximum light energy available from the blue-lamp system was 8.0×10^3 ergs cm^{-2} sec^{-1}. Higher energies (ergs) were available from the green and white light systems. However, most of our work was done with cultures grown at 7.9 to 8.0×10^3 ergs cm^{-2} sec^{-1}. The spectral distribution of the energy from each of the light sources is shown in Fig. 1.

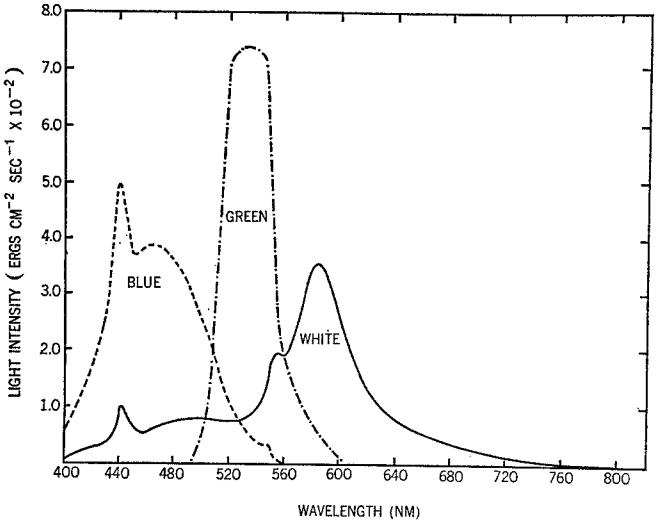

Fig. 1. Spectral distribution of the blue, green and white light systems used in this investigation. White light was obtained from cool white fluorescent lamps. Blue and green light were obtained from blue and green fluorescent lamps combined with sheets of blue or green celluloid, respectively

Results

Cultures were grown for 9 days under blue, green or white light before $^{14}CO_2$ fixation rates were measured Neutral density filters were used to achieve a range of desired intensities. Rates of photosynthesis by *Cyclotella nana* in relation to light quality and intensity are shown in Fig. 2A. Light saturation of photosynthesis was achieved around 5.0×10^3 ergs $cm^{-2}sec^{-1}$ in white or green light and 6.5×10^3 ergs cm^{-2} sec^{-1} in blue light. Rates of photosynthesis were higher in blue than in green or white light.

The energy produced by the blue light system was insufficient to saturate photosynthesis in *Dunaliella tertiolecta*. Therefore, light intensities were adjusted to obtain approximately the same number of quanta from each light source. The intensities varied from 7.4 to 8.0×10^3 ergs cm^{-2} sec^{-1}.

The rate of ^{14}C fixation by *Dunaliella tertiolecta* in blue light relative to white light was significantly higher than 1.0 ($p < 0.001$), while the green:white ratio was significantly lower than 1.0 ($p < 0.001$). These results, presented in Table 1, are similar to those for *Cyclotella nana* (Fig. 2).

Table 1. *Ratios of ^{14}C fixation by Dunaliella tertiolecta in blue or green light relative to white light at high light intensity*

Ratios of total fixation of ^{14}C	
Blue:white	Green:white
1.31	0.69
1.27	0.64
1.08	0.66
1.26	0.63

Table 2. *Ratios of ^{14}C fixation by Cyclotella nana and Dunaliella tertiolecta in blue or green light relative to white light at low light intensity*

Ratios of total fixation of ^{14}C			
Blue:white		Green:white	
C. nana	*D. tertiolecta*	*C. nana*	*D. tertiolecta*
1.81	1.29	0.61	0.48
1.74	1.23	0.63	0.39
1.79	1.21	0.68	0.41
—	1.26	0.65	0.46

The ratios of total ^{14}C-fixation in either blue or green light to that in white light after the cultures had been grown for 9 days in blue, green or white light at intensities approximately 10% of the intensities used in the first series of experiments, are presented in Table 2. The results were generally similar to those at high intensities, although the green:white ratios in *Dunaliella tertiolecta* were significantly lower in cells grown under low light intensities than those grown at high intensities ($p < 0.001$).

We also measured ^{14}C-fixation under light conditions approximating those near the bottom of the photic zone. The spectral distribution of available light

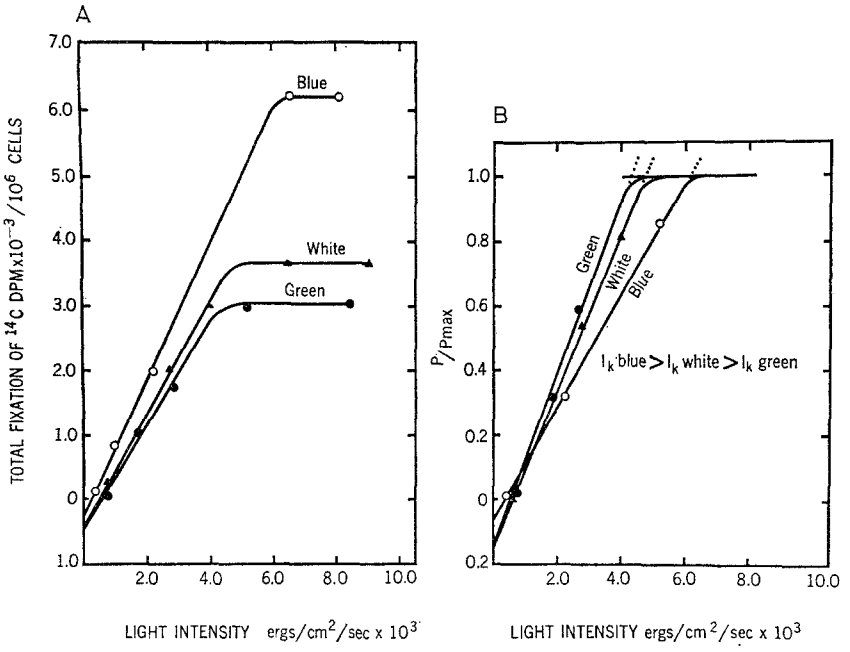

Fig. 2. *Cyclotella nana.* (A) Total fixation of ^{14}C after 30 min photosynthesis in blue, green or white light. (B) Normalized photosynthetic curves

was measured at 12 m depth in Indian Arm, a marine inlet in the vicinity of Vancouver, British Columbia, Canada. At this depth, approximately $^1/_4$ of the light was in the blue portion of the visible spectrum, the remainder was in the green part of the spectrum. Green and blue fluorescent bulbs and filters were combined to produce light of approximately similar composition. Light intensity was adjusted to approximately 10% of saturation intensities. The ^{14}C-fixation was measured and compared with the rates in white light (Table 3). The blue-green:white ratios of photosynthesis in *Cyclotella nana* and *Dunaliella tertiolecta* were similar to the blue:white ratios (Tables 1 and 2).

Table 3. *Ratios of ^{14}C fixation by Cyclotella nana and Dunaliella tertiolecta in a mixture of blue and green light to those in white light*

Ratios of total fixation of ^{14}C	
C. nana	*D. tertiolecta*
1.64	1.13
1.83	1.28
1.79	1.20
1.71	1.22

Cyclotella nana grew more rapidly in blue light than in either green or white light. Relative growth constants in the exponential phase were 0.37, 0.31 and 0.29, respectively (Fig. 3). Mean generation time varied between 44 and 65 h. When *C. nana* was

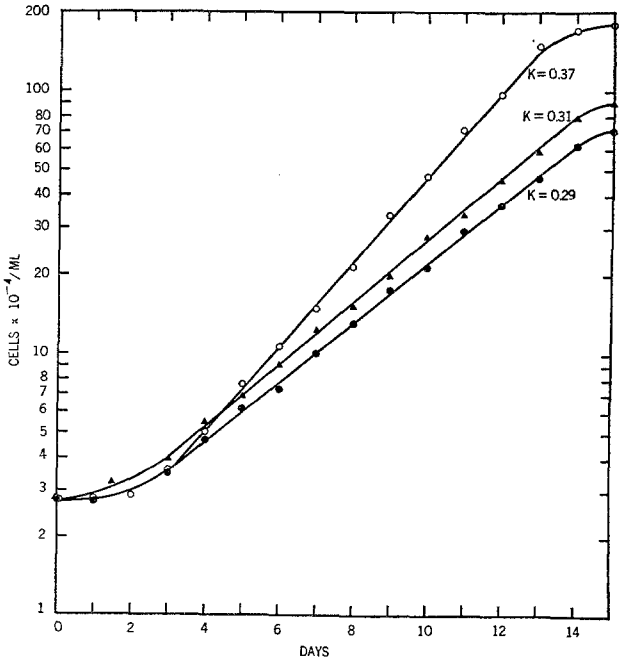

Fig. 3. *Cyclotella nana.* Growth in blue (open circles), green (black circles) and white light (triangles). Light intensity 8.0×10^3 ergs cm^{-2} sec^{-1}

removed from white light and put in either green or blue light, it grew more slowly at first and then attained a relatively constant growth rate which was higher in blue and lower in green light than in white light of similar intensity.

The relative growth constants of *Dunaliella tertiolecta* grown in blue, green, and white light were 0.41, 0.29 and 0.31, respectively (Fig. 4). Mean generation times varied from 40 to 57 h. *D. tertiolecta* transferred from white to blue light also exhibited a lag

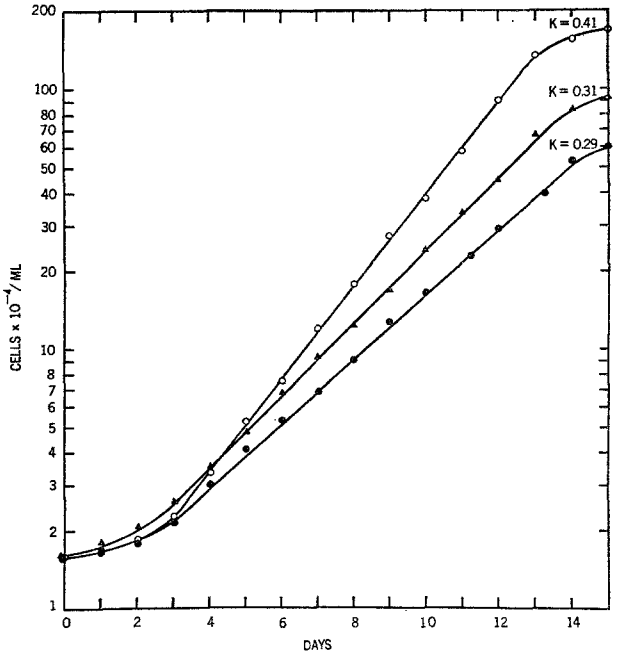

Fig. 4. *Dunaliella tertiolecta*. Growth in blue (open circles), green (black circles) and white light (triangles). Light intensity 8.0×10^3 ergs cm^{-2} sec^{-1}

before growing more rapidly than cells cultured in white light.

The distribution of ^{14}C in the various fractions in *Cyclotella nana* and *Dunaliella tertiolecta* grown in blue, green or white light is given in Table 4. When the cells were grown in blue or green light 65 to 70% of the radioactivity was in the ethanol-insoluble fraction compared with 10 to 30% in white light. This was observed at high or saturation light intensities and at intensities 10% of these levels. Conversely, the radioactivity incorporated into the ethanol-soluble fraction was relatively low in cells grown in blue or green light.

Some species differences in the distribution of ^{14}C were noted (Table 4). In white light, the ethanol-soluble fraction consistently contained 85 to 90% of the total ^{14}C assimilated by *Dunaliella tertiolecta* and 65 to 70% of the ^{14}C assimilated by *Cyclotella nana*. These species differences were not apparent in blue or green light.

Hydrolysis of the ethanol-insoluble fraction showed that protein amino acids account for about 96% of the ^{14}C fixed in this fraction in blue or green light (Table 5). In white light the protein amino acids account for 92% of the total radioactivity.

The distribution of ^{14}C within the ethanol-soluble fraction is shown in Table 6. The main ethanol-soluble non-nitrogenous compound in *Dunaliella tertiolecta* is glycerol. Glucose and trace quantities of sucrose also occur. Glucose is the primary soluble carbohydrate in *Cyclotella nana*. Trace quantities of fructose, ribose and mannose and one unknown consistently appeared.

Table 4. *Distribution of ^{14}C among the ethanol-soluble and ethanol-insoluble fractions in Cyclotella nana and Dunaliella tertiolecta*

Organism	Spectral quality of light	Total fixation of $^{14}C/10^9$ cells (μCi)	Radioactivity as % of the total ^{14}C fixed	
			Ethanol-insoluble fraction (%)	Ethanol-soluble fraction (%)
High intensity				
C. nana	white	12.8	32.7	67.3
	blue	22.4	64.1	35.9
	green	11.0	60.2	39.8
D. tertiolecta	white	19.0	10.1	89.9
	blue	24.6	71.2	28.8
	green	12.4	66.8	33.2
Low intensity				
C. nana	white	3.5	33.4	66.6
	blue	6.2	61.4	38.6
	green	3.2	59.1	40.9
D. tertiolecta	white	5.9	13.8	86.2
	blue	7.4	72.5	27.5
	green	2.6	69.3	30.7

When *Cyclotella nana* and *Dunaliella tertiolecta* were exposed to blue light, there was an increase in the radioactivity of aspartic acid, glutamic acid, asparagine, glutamine, serine, alanine, malic acid and fumaric acid relative to that observed in white light. Radioactivity decreased in glycine, glucose and the phosphate esters. *D. tertiolecta* differed in that no radioactivity was detected in glutamine after 30 min of photosynthesis. The radioactivity in glycerol decreased in blue light compared with white light.

When *Cyclotella nana* and *Dunaliella tertiolecta* were incubated in green light, the quantity of serine, aspartic, glutamic, malic, and fumaric acids increased relative to white light; the concentration

Table 5. *Cyclotella nana and Dunaliella tertiolecta. Total $^{14}C/10^9$ cells in the ethanol-insoluble fraction and in the products resulting from hydrolysis of this fraction*

Organism	Light quality	Ethanol-insoluble fraction	Protein-amino acids		Carbohydrates		Unrecovered
		(μCi)	(μCi)	(%)	(μCi)	(%)	(%)
C. nana	white	4.2	3.8	92.6	0.22	5.4	2.0
	blue	14.2	13.5	95.3	0.04	1.3	3.4
	green	6.6	6.5	98.8	0.10	1.2	—
D. tertiolecta	white	1.9	1.7	92.0	0.12	6.8	2.2
	blue	17.5	16.8	96.1	0.20	1.3	2.6
	green	8.3	7.9	95.9	0.15	1.8	2.3

Table 6. *Distribution of ^{14}C among compounds of the ethanol-soluble fraction in Cyclotella nana and Dunaliella tertiolecta exposed to high light intensities*

	% of total ^{14}C fixed in the ethanol-soluble fraction											
	(A)						(B)					
Organism	*C. nana*			*D. tertiolecta*			*C. nana*			*D. tertiolecta*		
Light quality	white	blue	green	white	blue	green	white	blue	green	white	blue	green
Aspartic acid	12.6	16.5	17.3	13.2	16.4	17.1	12.3	15.9	16.1	11.1	15.9	16.5
Glutamic acid	2.8	6.6	6.1	1.7	4.4	4.9	3.2	7.1	5.8	2.3	4.2	4.8
Asparagine	1.0	2.2	1.1	0.9	1.4	0.9	0.8	3.1	3.1	1.1	1.5	1.3
Glutamine	0.5	1.3	0.6	—	—	—	1.0	1.6	1.1	—	—	—
Glycine	0.9	0.5	0.8	13.2	11.5	11.9	3.1	2.1	2.3	12.8	10.9	11.4
Serine	7.5	8.2	8.6	8.1	10.0	9.8	8.1	10.2	9.3	7.3	10.1	9.4
Alanine	3.5	5.0	3.5	6.8	9.4	8.0	6.1	8.7	8.9	9.2	10.9	11.1
Malic acid	11.4	16.6	17.2	6.8	8.0	8.6	9.3	12.8	13.2	6.9	7.9	8.2
Fumaric acid	3.0	3.9	3.8	1.4	2.4	2.8	2.9	3.7	4.1	1.9	2.8	2.6
Glycolic acid	4.4	2.0	2.9	6.2	4.1	5.8	6.2	4.1	5.3	4.1	3.9	2.8
Succinic acid	0.8	0.7	—	1.0	1.0	0.9	—	0.4	0.8	1.1	—	1.1
Glucose	18.3	15.2	15.4	1.2	1.4	—	14.5	11.6	12.1	2.1	1.3	1.5
Glycerol	—	—	—	12.9	7.7	7.9	—	—	—	14.3	10.4	9.9
Phosphate esters	33.3	21.3	22.0	26.6	22.3	21.4	32.5	18.7	18.1	25.8	20.3	19.4

Cell number approximately (A) 3×10^5 cells/ml; (B) 1×10^3 cells/ml.

Table 7. *Distribution of ^{14}C among compounds of the ethanol-soluble fraction of Cyclotella nana or Dunaliella tertiolecta exposed to low light intensities*

	(A) % of total ^{14}C fixed in the ethanol-soluble fraction						(B) % of total ^{14}C fixed in the ethanol-soluble fraction					
Organism	*C. nana*			*D. tertiolecta*			*C. nana*			*D. tertiolecta*		
Light quality	white	blue	green	white	blue	green	white	blue	green	white	blue	green
Aspartic acid	12.1	15.9	15.7	11.3	15.2	15.3	13.7	18.0	17.3	11.9	16.1	14.8
Glutamic acid	5.2	9.4	9.7	2.5	4.1	3.8	8.0	11.3	10.6	1.3	4.2	4.4
Asparagine	0.9	2.8	1.2	—	1.0	0.8	2.2	3.1	3.4	1.0	2.1	1.4
Glutamine	0.6	1.5	0.9	—	—	—	0.9	2.0	2.8	—	—	—
Glycine	1.1	1.9	2.2	12.9	10.3	11.5	4.0	1.9	1.5	13.8	12.0	11.7
Serine	8.6	9.9	9.2	7.5	9.9	10.1	5.8	7.3	6.9	8.8	10.3	9.9
Alanine	5.1	8.3	8.5	7.3	8.1	7.9	9.0	11.1	11.0	6.1	8.9	9.1
Malic acid	10.9	13.7	14.3	6.2	8.3	8.2	8.2	12.4	11.9	7.3	8.3	7.9
Fumaric acid	3.2	4.2	4.0	2.1	3.0	3.0	2.6	3.7	4.3	1.1	1.9	1.3
Glycolic acid	5.6	2.1	3.2	5.1	4.1	4.4	4.2	3.8	3.5	5.3	5.8	4.1
Succinic acid	0.9	0.7	0.9	1.0	1.0	—	1.1	1.0	1.1	0.5	—	0.9
Glucose	16.6	8.6	9.3	2.1	1.1	—	15.6	12.1	12.6	1.8	0.7	1.5
Glycerol	—	—	—	13.2	8.1	7.7	—	—	—	15.0	9.6	10.7
Phosphate esters	29.3	21.1	20.9	28.7	25.7	26.1	24.7	12.3	13.1	26.1	20.1	22.3

Cell numbers approximately (A) 3×10- cells/ml; (B) 1×10^3 cells/ml.

of glucose in *C. nana*, glycerol in *D. tertiolecta*, and phosphate esters and glycine decreased.

We conducted a series of experiments at light intensities approximately 10% of those used in the initial experiments. The nature of the photosynthate in *Cyclotella nana* and *Dunaliella tertiolecta* grown at intensities approximately 10% of those used in the earlier experiments was similar to that in cells grown at saturating or high intensities of the same light (Table 7). This suggests that the effect of light quality on the nature of the photosynthate is independent of the photosynthetic rate. Furthermore, cell concentration did not have an important influence on the distribution of compounds in the ethanol-soluble fraction.

Discussion

Growth, photosynthetic rate and the nature of the photosynthate in planktonic algae changes in response to variations in a number of environmental factors such as temperature, CO_2 concentration, salinity, and light intensity. Our results for *Cyclotella nana* and *Dunaliella tertiolecta* (Figs. 2 to 4, Tables 1 to 3) indicate a response to the spectral quality of available light. These data also reveal low growth rates and generation times in our cultures relative to those reported by other workers for enriched cultures of plankton algae. Our cultures were grown in artificial sea water which permitted doubling every 1 or 2 days, a rate not unlike those reported in natural populations.

The growth rates in our cultures were consistently highest in those cultures grown under blue light and lowest in green light cultures. Our results are not in agreement with those of BAATZ (1941). She reported that growth of cells in green light was significantly higher than in blue light. It may be that these differences are attributable to species characteristics or to differences in the spectral composition of the energy sources used in the two studies.

We considered the effects of light quality on the light intensity-photosynthesis curves (Fig. 2). Maximum rates of photosynthesis are lower in green and white than in blue light. To permit comparison of curves without the distortion of differences in the overall magnitude of photosynthesis, we have normalized the curves and determined I_k values (TALLING, 1957) which represent the ratio between the rate of photochemical processes in photosynthesis per unit energy and the maximum rate of enzymatic processes. The I_k values varied from 6.2×10^3 ergs cm^{-2} sec^{-1} in blue light to 4.7×10^3 and 4.2×10^3 ergs cm^{-2} sec^{-1} in white and green light, respectively (Fig. 2B). The higher I_k and light-saturated rate of photosynthesis in blue light suggests that concentrations of photosynthetic enzymes are probably higher in blue light cells. Furthermore, the slope of the linear portion of the curves in Fig. 2A suggests that *Cyclotella nana* cells grown in blue light contain a higher concentration of photosynthetic pigments than those from green and white light. This was verified by direct measurements. When the curves in Fig. 2A are normalized (Fig. 2B), the white and green light curves are shifted to the left of the blue light curves. This suggests that, relative to blue light, the dark reactions in green and white light were affected to a greater degree than the light reactions (YENTSCH and LEE, 1966).

There are numerous examples of environmental parameters which have a greater effect on dark reactions than light reactions. Lower temperature, CO_2, nutrient concentrations and light intensity all affect dark reactions to a greater degree than light reactions (RABINOWITCH, 1951; STEEMANN NIELSEN and HANSEN, 1961; YENTSCH and LEE, 1966). YENTSCH and LEE also suggested that lower I_k values, such as those observed when *Cyclotella nana* was grown in white or green light, is a response to a physiologically inferior environment. However, this does not preclude the possibility that adaptation (as defined by STEEMANN NIELSEN and JØRGENSEN, 1968) occurs. Our results indicate that algae adapt to light quality, although the extent of adaptation is not sufficient to equalize the quantum efficiency of photosynthesis in the 3 light systems.

The relative respiration rates in *Cyclotella nana* and *Dunaliella tertiolecta*, estimated by the method of STEEMANN NIELSEN and HANSEN (1959), were lower in blue light cells than in those grown in green or white light (Fig. 2A). Published data on the effect of light quality on respiration are sparse. KOWALLIK (1967) reported that blue light stimulated respiration in algae. In contrast, HUMPHREY and SUBBA RAO (1967) reported similar ratios of photosynthesis to respiration in blue and white light for the diatom *Cylindrotheca closterium*. The lower respiration and higher photosynthetic rate in *C. nana* and *D. tertiolecta* cells grown in blue light will result in incorporation of a greater proportion of assimilated CO_2 into cellular material than in comparable cells grown in either white or green light. Hence, algal cells grown under blue light will reach division size faster and should have a higher growth rate. This is supported by data in Figs. 3 and 4.

The enhanced photosynthesis in blue light would counteract, in part, the effect on photosynthesis of the low intensities encountered in the lower part of the photic zone in the open ocean. It would also increase the compensation depth for algae.

The ratio of ^{14}C uptake in algae grown in blue-green light (Table 3) was similar to the blue:white ratio (Table 2) suggesting that the blue light overrides the effect of green light. Otherwise a lower blue-green:white ratio might have been expected. This may be due to the efficient absorption of photons in the blue portion of the spectrum by chlorophyll. Only the accessory pigments such as carotenoids can utilize light in the green portion of the spectrum. However,

TANADA (1951) reported that the photosynthetic efficiency of diatom carotenoids was almost as high as chlorophyll. The carotenoids of green algae are only half as efficient as chlorophyll (EMERSON and LEWIS, 1943). Our data suggest a low carotenoid efficiency in cells exposed to a mixture of blue and green light. We are unable to explain the mechanism involved. However, this type of response is not new. HAUSCHILD et al. (1962a) observed that, in photosynthesizing *Chlorella vulgaris*, an addition of as little as 4% blue radiation to red light had the same metabolic effect as irradiation with blue light alone.

Light quality had a marked effect on the distribution of ^{14}C in algal photosynthate. Two major changes were observed in the distribution of ^{14}C in *Cyclotella nana* and *Dunaliella tertiolecta* grown in blue or green light compared with white light (Table 4): (1) In blue or green light, 60 to 70% of the total ^{14}C fixed by the cells was located in the ethanol-insoluble fraction, whereas, in white light, the ethanol-insoluble fraction contained from 10 to 30% of the fixed ^{14}C. (2) The spectral composition of light had a marked effect on the relative importance of components within the ethanol-soluble fraction, but not within the ethanol-insoluble fraction.

The results from hydrolysis of the ethanol-insoluble fraction are given in Table 5. A minimum of 92% of the activity was recovered on hydrolysis of proteins and a further 1 to 7% was recovered as sugars. Of the 16 to 18 common protein amino acids, aspartic acid, glutamic acid and alanine contained most ^{14}C activity (Table 8). This agrees with the results reported for several chlorophycean and rhodophycean algae (CRAIGIE et al., 1966; MAJAK et al., 1966). While protein formation is enhanced in blue or green light, light quality has no effect on the relative distribution of ^{14}C among the protein amino acids. Thus, it appears that shorter wavelength light [defined for the purposes of this discussion as the wavelengths available from the blue and green light systems (400 to 580 nm)] changes the rate of protein synthesis but not its direction. This agrees with the results of VOSKRENSENSKAYA (1956) for bean and tobacco leaves, but contrasts with the results of HAUSCHILD et al. (1962a, b), who did not demonstrate any differential effect of blue light on the incorporation of ^{14}C into the protein of several freshwater algae.

Glucose, probably derived from the hydrolysis of starch, was the most significant radioactive sugar recovered from *Dunaliella tertiolecta* on carbohydrate hydrolysis. Glucose and small quantities of mannose were recovered from *Cyclotella nana*. These two sugars may have been derived from a substance similar to laminarin, a reserve polysaccharide which occurs in brown algae (BEATTIE et al., 1961), or from cell-wall polysaccharides.

The ethanol-soluble fraction in cells grown in blue or green light contained a higher proportion of ^{14}C in alanine, serine, aspartic, glutamic, fumaric and malic acids than those grown in white light. Conversely, the relative importance of glycine and carbohydrates was reduced. These results were observed in cells grown under high or low light intensities. Apparently, the changes in distribution of ^{14}C among the photosynthetic products are not dependent upon rates of photosynthesis, but are associated with differences in light quality. Short wavelength light stimulates protein synthesis at the expense of ethanol-soluble compounds such as carbohydrates and glycine.

If the concentration of free amino acids is expressed as a percent of the total ^{14}C fixed by the cells (Table 9), the amino acid pools are smaller in cells grown under blue or green light than white light. This is consistent with the enhanced protein formation observed under short wavelength light. The increase in amino acids required for protein synthesis, in particular glutamate and aspartate, would deplete the supply of oxaloacetate and other Krebs cycle intermediates. If amino acids are being synthesized from these intermediates under short wavelength light, then these intermediates must be replaced to ensure functioning of the Krebs cycle. This could be achieved by β-carboxylation of pyruvate or phosphoenol pyruvate (PEP) to give oxaloacetate or malate. WALKER (1962) suggested that β-carboxylation reactions may account for a significant proportion of the total carbon fixed in light. Recently, BALDRY et al. (1969) reported a light stimulated β-carboxylation of PEP by isolated chloroplasts. Alternatively, it may be that short wavelength light favors the Hatch-Slack pathway of CO_2 fixation, resulting in

Table 8. *Distribution of ^{14}C among the protein amino acids after photosynthesis by Cyclotella nana and Dunaliella tertiolecta*[a]

Amino acid	% of total ^{14}C fixed in the protein fraction					
	C. nana			*D. tertiolecta*		
	white	blue	green	white	blue	green
Aspartate	13.9	14.3	14.8	14.5	15.1	14.7
Glutamate	18.1	17.8	18.6	17.5	16.9	16.9
Alanine	15.3	14.9	15.0	16.0	15.9	16.3
Glycine	6.6	6.0	6.3	6.4	7.1	7.0
Serine	3.4	3.0	2.7	5.3	5.8	5.1
Leucine	7.5	8.0	7.2	6.7	7.0	6.3
Proline	4.9	4.3	4.8	5.4	5.9	5.2
Phenylalanine	8.5	8.8	8.8	3.3	2.7	2.9
Threonine	5.8	6.2	6.0	6.2	7.0	6.8
Tyrosine	3.7	3.8	3.6	2.2	2.1	2.6
Valine	0.6	0.8	0.5	3.8	3.3	3.9
Methionine	4.9	5.3	5.1	2.5	3.1	2.9
Ornithine	0.4	0.5	0.8	—	—	—
Arginine	3.2	4.0	3.7	—	—	—
Unknown	3.6	3.0	3.1	10.2	8.1	9.4

[a] Approximately 3×10^5 cells/ml.

an increased production of oxaloacetate, malate and other Krebs cycle intermediates (HATCH and SLACK, 1966). The occurrence of this pathway in algae has not yet been demonstrated, nor are the possible effects of light of different wavelengths known. In the Hatch-Slack pathway, the label in the C_4-dicarboxylic acids appears in 3-phosphoglycerate, then hexose phosphates and other carbohydrates. Hence, it is doubtful that this is the mechanism involved in these two algae, since blue and green light promote the synthesis of proteins at the expense of carbohydrates. Thus, our data do not identify the reactions involved in CO_2 fixation, but they do show that short wavelength light alters the direction of algal metabolism in favor of protein synthesis in *Cyclotella nana* and *Dunaliella tertiolecta*, while white light promotes the formation of carbohydrates.

Table 9. *Distribution of ^{14}C among compounds of Cyclotella nana or Dunaliella tertiolecta as a percentage of the total ^{14}C fixed*

Organism	% of total ^{14}C fixed					
	C. nana			*D. tertiolecta*		
Light quality	white	blue	green	white	blue	green
Aspartic acid	7.6	5.4	6.3	11.3	4.6	5.4
Glutamic acid	1.7	2.2	2.2	1.4	1.2	1.5
Asparagine	0.6	0.7	0.4	0.8	0.4	0.3
Glutamine	0.3	0.4	0.2	—	—	—
Glycine	0.5	0.2	0.3	11.3	3.2	3.7
Serine	4.5	2.7	3.1	6.9	2.8	3.1
Alanine	2.1	1.6	1.3	5.8	2.6	2.5
Malic acid	6.9	5.4	6.3	5.8	2.2	2.4
Fumaric acid	1.8	1.3	1.4	1.2	0.7	0.9
Glycolic acid	2.6	0.7	1.1	5.3	1.2	1.8
Succinic acid	0.5	0.2	—	0.9	0.3	2.8
Succinic acid	0.5	0.2	—	0.9	0.3	2.8
Glucose	11.0	5.0	5.6	1.0	0.4	—
Glycerol	—	—	—	10.9	2.2	2.5
Phosphate esters	20.0	6.9	8.0	22.7	6.3	6.7
Total amino acids	17.3	13.1	13.8	37.4	14.9	16.5
Total organic acids	11.8	7.6	8.7	12.6	4.3	5.7
Total carbohydrates	31.0	11.9	13.6	34.7	8.8	9.2
Total protein amino acids	29.7	60.3	59.1	8.9	67.4	63.7
Total hydrolized carbohydrates	1.7	0.2	0.9	0.6	0.8	1.2

Our data indicate that both the rate of CO_2 fixation per unit energy and the nature of the photosynthetic products in algae would be changed as light quality is altered with depth in the photic zone. The enhanced photosynthesis in blue or blue-green light has the effect of maximizing the capacity of plankton algae to utilize the light available in the lower part of the photic zone.

Summary

1. Rates of photosynthesis, growth and I_k values in *Cyclotella nana* (HUSTEDT) and *Dunaliella tertiolecta* (BUTCHER) were higher in blue light than in white or green light of equal intensities. These differences in photosynthetic rate and growth were observed at both high and low intensities.

2. Relative growth constants in blue, white and green light were 0.37, 0.31 and 0.29 for *C. nana*, and 0.41, 0.31 and 0.29 for *D. tertiolecta*.

3. Light quality influenced the distribution of newly incorporated ^{14}C. In blue or green light 65 to 70% of the radioactivity was in the ethanol-insoluble fraction. From 10 to 30% of the activity was in this fraction in cells grown in white light. Conversely, radioactivity incorporated into the ethanol-soluble fraction was low in cells grown in blue or green light and high in white light.

4. Hydrolysis of the ethanol-insoluble fraction yielded a minimum of 92% of the activity in protein amino acids. Although the relative rate of protein synthesis was enhanced in blue or green light, there was no apparent effect of light quality on the distribution of ^{14}C among the protein amino acids.

5. The ethanol-soluble fraction from cells grown in blue or green light contained a higher proportion of ^{14}C in alanine, serine, aspartic, glutamic, fumaric and malic acids than the same fraction in white light.

6. Blue or green light influenced algal metabolism in favor of protein synthesis; white light caused carbohydrate synthesis. This suggests that the chemical composition of natural phytoplankton populations varies with depth.

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