THE EFFECT OF LIGHT QUALITY ON GROWTH RATES, PHOTOSYNTHETIC RATES AND METABOLISM IN PLANKTON ALGAE

by

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TO DONNA JEAN AND TRISTAN

"Yes", I answered you last night;
"No", this morning sir, I say:
Colours seen by candle-light
Will not look the same by day.

Elizabeth Barrett Browning

ABSTRACT

The effect of light quality on growth, photosynthesis, pigment concentrations and carbon metabolism in two species of marine algae, Cyclotella nana and Dunaliella tertiolecta was examined. Relative growth constants for Cyclotella 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 Dunaliella. Photosynthetic rates of the two species were higher in blue and lower in green than in white light of the same intensity.

More than 60% of the ^{14}C assimilated by Cyclotella or Dunaliella grown in blue or green light was incorporated into the ethanol-insoluble fraction, compared with 10-30% 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 rates and densities of cells in the cultures.

The concentrations of chlorophyll a and b or c in

Dunaliella or Cyclotella were higher under blue light and lower under green than white light of the same intensity. Total carotenoid concentrations were higher in green and lower in blue light.

RNA and DNA concentrations were higher in blue and lower in green than in white light. Similar variations were observed in the concentration of total cell protein. Turnover of recently formed protein was indicated.

Variations in the distribution of photosynthetically fixed ^{14}C in phytoplankton growing at different depths in Saanich Inlet and Indian Arm, B. C. were examined. Generally, surface phytoplankton contained the greatest proportion of ^{14}C in the ethanol-soluble fraction irrespective of light intensity. Near the bottom of the photic zone, the greatest proportion of newly formed compounds were in the insoluble fraction. The various components of the ethanol-soluble fraction changed with depth within the photic zone. Carbohydrates decreased with depth and amino acids increased with depth. These shifts appear to be a response to changes in light quality.

The percentage release of soluble organic carbon by phytoplankton was highest at the surface and was proportional to the size of the ethanol-soluble fraction.

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Chapter 1

LIGHT QUALITY EFFECTS ON GROWTH, PHOTOSYNTHETIC RATES AND CARBON METABOLISM IN TWO SPECIES OF MARINE PLANKTON ALGAE

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 Krotkov 1964, Strickland 1960). 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. 1953) have a demonstrable effect on chemical composition. The wavelength of light 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, 1962b). Further, light quality produced 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, 1962b), Hess and Tolbert (1967), and Zak (1965), reported that light quality influenced 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 since light of the various wavelengths is differentially attenuated. Thus, blue and green light penetrate to 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, as an example, result in a blue light enhancement of protein synthesis. The nature of the photosynthate may, in turn, influence the quantity and quality of soluble organic compounds the algae excrete into the environment. These excreted compounds may be of considerable ecological significance as a source of energy for heterotrophic organisms or in the formation of organic aggregates.

In this section the effects of blue, green and white light on photosynthetic rates, growth rates, and the nature of the photosynthate in two marine planktonic algae, Cyclotella nana Hustedt and Dunaliella tertiolecta Butcher were examined.

MATERIALS AND METHODS

Axenic cultures of two marine algae, the diatom Cyclotella nana and the green alga Dunaliella tertiolecta 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 of vitamin solution containing 500 μg/ml thiamine-HCl, 2 μg/ml vitamin B_{12} and 1 μg/ml of biotin.

In addition, 0.15 g/l $NaSiO_3.H_2O$ were added to the medium in which diatoms were cultured. The same volume of 0.01 N HCl as that of silicate was added to maintain a pH of 7.5 - 7.6. The medium was sterilized by autoclaving for 20 min at 120 C and 15 psi (Appendix I).

Growth and Photosynthetic Rate 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 of medium. Stock cultures were maintained in white light in screw-capped 125-ml Erlenmeyer flasks containing 50 ml of the same medium. Routine checks of both stock and experimental cultures verified the absence of contamination (Appendix II). 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 1-3 x 10^4 cells/ml and the cultures were placed under the appropriate light conditions.

Growth rates were determined 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: --

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

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

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

To measure photosynthetic rates, 100 μCi of $^{14}CO_3^{2-}$ were added to each of several replicates of 200 ml of cell suspension taken from the log phase of growth. At end of the experiment cell numbers were determined and an aliquot from each suspension was filtered through a Millipore HA filter (47 mm, pore diam. 0.45μ). Filtration removed dissolved salts which interfere with ion-exchange separation and chromatography of the ethanol-soluble components. The cells were killed and extracted in boiling 80% ethanol for 1 hr. The ethanol-insoluble fraction was then removed with a Millipore filter. The filtrate was acidified with 0.1 N HCl to pH 3 and aerated vigorously for 1 hr to remove $^{14}CO_2$. The ethanol-soluble extracts were reduced to a small volume <u>in vacuo</u> 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 20 hr 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 of distilled water. A 10 μl aliquot from the ethanol-soluble fraction was assayed for ^{14}C activity. The amino acid, organic acid, and sugar fractions were separated on Rexyn 101 (H^+) and Rexyn 201 ($HCOO^-$) ion exchange columns. More than 96% of the total radioactivity present in the ethanol-soluble extracts was recovered in these three 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 (267:37:1) and n-propanol:ethyl acetate:water (7:12:2). 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.

Later, a second two-dimensional system was used which gave better separation of these amino acids. The first solvent system consisted of phenol:ammonium hydroxide:water (300:75:1)

and the second solvent system consisted of butanol:acetone: diethylamine:water (11:11:2:5).

The organic acid chromatograms were developed in two dimensions with ethanol:ammonium hydroxide (95:5) and the organic phase of butanol:formic acid:water (4:1:5). This solvent system was prepared 12 hr 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) and ethyl acetate:acetic acid:water:sodium acetate (20:11:10:48).

Chromatograms were exposed to Ilford Ilfex X-ray film for 1 - 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 scintillation cocktail. The efficiency of paper spot counting was determined 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.

Resolved compounds 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.

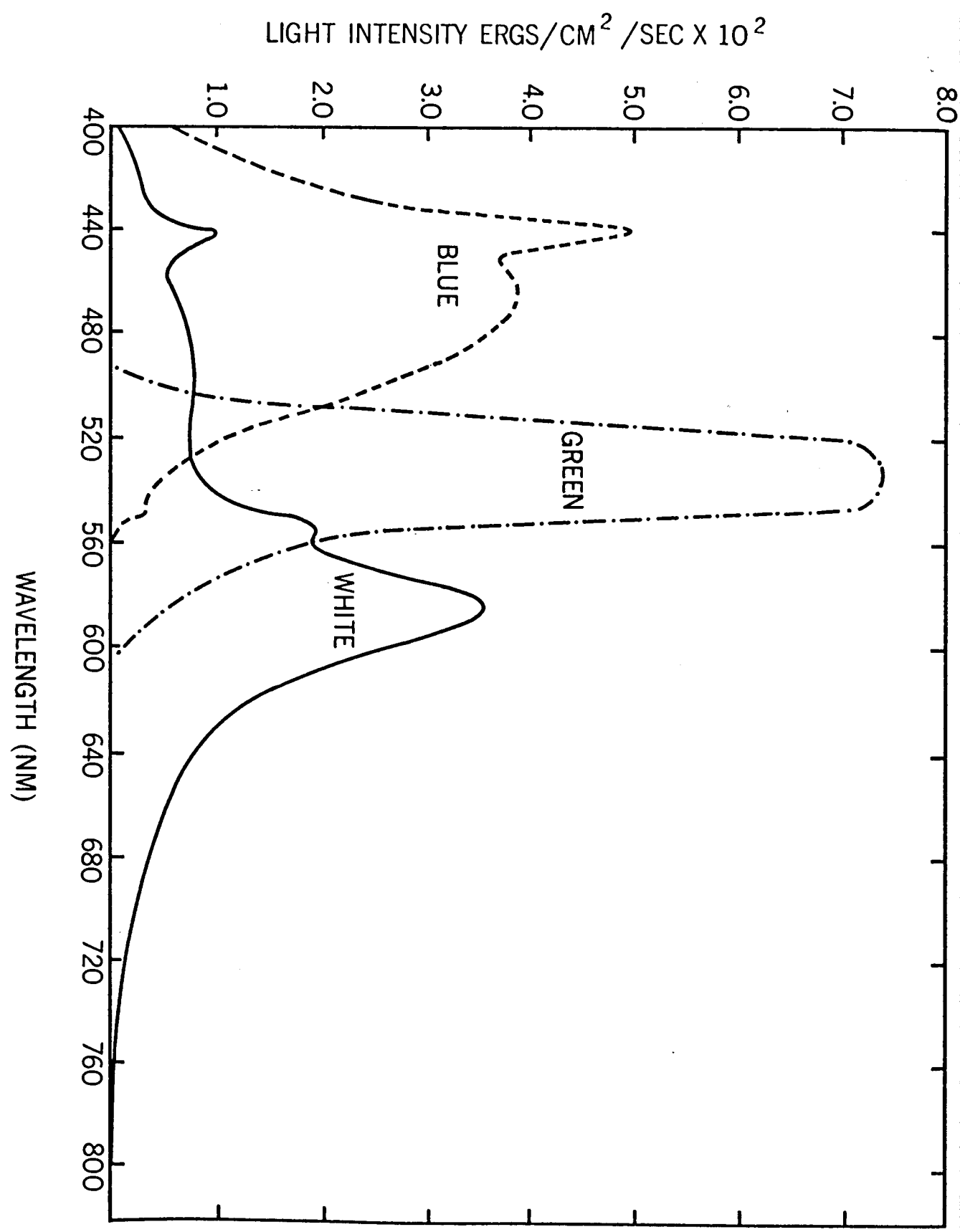
The ethanol-insoluble residues were converted to CO_2 by the wet combustion method described by Hofstra (1967) to

measure total ^{14}C fixed in this fraction. In addition, in some experiments, aliquots of the ethanol-insoluble residues were hydrolyzed in a Soxhlet apparatus. One portion was hydrolyzed in 1 N H_2SO_4 for 15 hr at 105 C, the other hydrolyzed in 6 N HCl for 20 hr 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 hr. The cells were illuminated from above with white, blue or green fluorescent lamps. In the latter two cases sheets of blue or green celluloid respectively, used as filters, were placed between the lamps and the culture vessels. The energy and wavelength of light at the surface of the cultures were measured with an ISCO Spectroradiometer. Maximum output was 7.9×10^3 ergs cm^{-2} sec^{-1} from the white lamps and 8.0×10^3 ergs cm^{-2} sec^{-1} from the blue or green lamps. The spectral distribution of the various lamps and filters is

Figure 1.1 Spectral distribution of the three light systems used in this investigation. White light was obtained from cool white fluorescent lamps. Blue or green light was obtained by combining sheets of blue or green celluloid with blue or green fluorescent bulbs. Maximum energy output was 7.9×10^3 ergs cm^{-2} sec^{-1} from the white lamps and 8.0×10^3 ergs cm^{-2} sec^{-1} from the blue or green lamps.


LIGHT INTENSITY ERGS/CM^2 /SEC X 10^2
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
400
440
480
520
560
600
640
680
720
760
800
BLUE
GREEN
WHITE
WAVELENGTH (NM)

shown in Fig. 1.1.

RESULTS

Rate of $^{14}CO_2$-fixation

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. 1.2A. 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 at saturation intensities 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. Therefore, light intensities were adjusted to obtain approximately the same number of quanta from each light source. The intensities varied from $7.4 - 8.0 \times 10^3$ ergs cm^{-2} sec^{-1}.

The rate of ^{14}C fixation by Dunaliella 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

Figure 1.2 A. Total fixation of ^{14}C by Cyclotella nana after 30 min photosynthesis in blue, green or white light.

B. Normalized photosynthetic curves. The data were taken from Figure 1.2A. I_k represents the ratio between the rate of photochemical processes (P) in photosynthesis and the maximum rate of enzymatic processes (P_{max}).

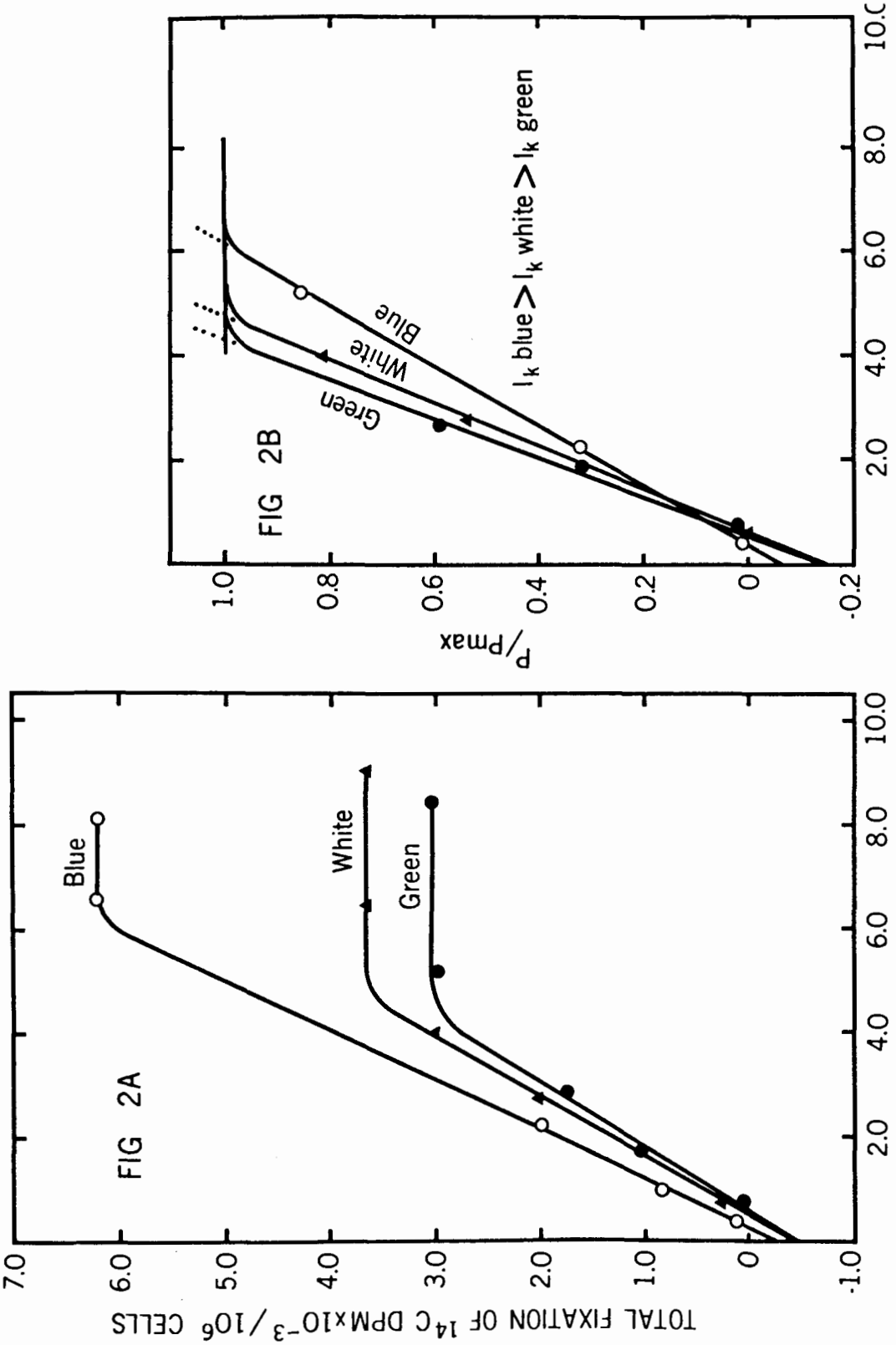


FIG 2A
Blue
White
Green
TOTAL FIXATION OF ^{14}C DPMx10^{-3}/10^6 CELLS
LIGHT INTENSITY ergs/cm^2/sec x 10^3
FIG 2B
I_k blue > I_k white > I_k green
P/Pmax
LIGHT INTENSITY ergs/cm^2/sec x 10^3

Table 1.1 are similar to those for Cyclotella (Fig. 1.2A).

Table 1.2 shows the ratios of total ^{14}C-fixation in either blue or green light to that in white light after the cultures have 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. The results were generally similar to those at high intensities although the green:white ratios in Dunaliella 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 was measured at 12 m depth in Indian Arm, a marine inlet in the vicinity of Vancouver, B. C. At this depth, approximately one-quarter 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 the saturation intensities. The ^{14}C-fixation was measured and compared with the rates in white light (Table 1.3). The blue-green:white ratios of photosynthesis in Cyclotella and Dunaliella were similar to the blue:white ratios.

Table 1.1

Ratios of ^{14}C fixation by Dunaliella tertiolecta in blue or green light relative to white light. Cells were preconditioned to a 16:8 hr light:dark cycle. $^{14}CO_2$ was added 30 min after the beginning of the light period. Experimental time was 30 min. Light intensities were varied within the range 7.3×10^3 ergs cm^{-2} sec^{-1} to 8.0×10^3 ergs cm^{-2} sec^{-1} to give approximately the same number of quanta.

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 1.2

Ratios of ^{14}C fixation by Cyclotella nana and Dunaliella tertiolecta in blue or green light relative to white light. Light intensities were varied within the range 7.9×10^2 - 8.5×10^2 ergs cm^{-2} sec^{-1} to give approximately 10% of the intensities used in the experiments reported in Table 1.1. Cells were preconditioned to a 16:8 hr light:dark cycle. $^{14}CO_2$ was added 30 min after the beginning of the light period. Experimental time was 30 min.

Ratios of total fixation of ^{14}C			
Cyclotella	Dunaliella	Cyclotella	Dunaliella
Blue:white		Green:white	
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

* Lost

Table 1.3

Ratios of ^{14}C fixation by Cyclotella nana and Dunaliella tertiolecta in a mixture of blue and green to that in white light. Cells were preconditioned to a 16:8 hr light:dark cycle. $^{14}CO_2$ was added 30 min after beginning of new light period. Experimental time was 30 min. Light intensity was 7.9×10^2 ergs cm^{-2} sec^{-1}.

Ratios of total fixation of ^{14}C	
Cyclotella nana	Dunaliella tertiolecta
1.64	1.13
1.83	1.28
1.79	1.20
1.71	1.22

Growth of Cyclotella nana and Dunaliella tertiolecta

Cyclotella grew more rapidly in blue light than in either green or white light. Relative growth constants in the exponential phase were 0.37, 0.25 and 0.29 respectively (Fig. 1.3). The mean generation time for Cyclotella varied between 44 - 65 hr, depending on the spectral quality of the available light. When Cyclotella was 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 when grown in blue, green, and white light were 0.41, 0.29 and 0.31 respectively (Fig. 1.4). Mean generation times varied from 40 - 57 hr. Dunaliella transferred from white to blue light also exhibited a lag before growing more rapidly than cells cultured in white light.

Effect of light quality on distribution of ^{14}C in algal photosynthate

The distribution of ^{14}C in the various fractions in Cyclotella and Dunaliella grown in blue, green or white light is given in Table 1.4. When the cells were grown in blue or green light 65 - 70% of the radioactivity was in the

Figure 1.3 Growth of Cyclotella nana under saturating intensities of blue (o), green (●) or white light (Δ). Light intensity 7.9×10^3 ergs cm^{-2} sec^{-1}.

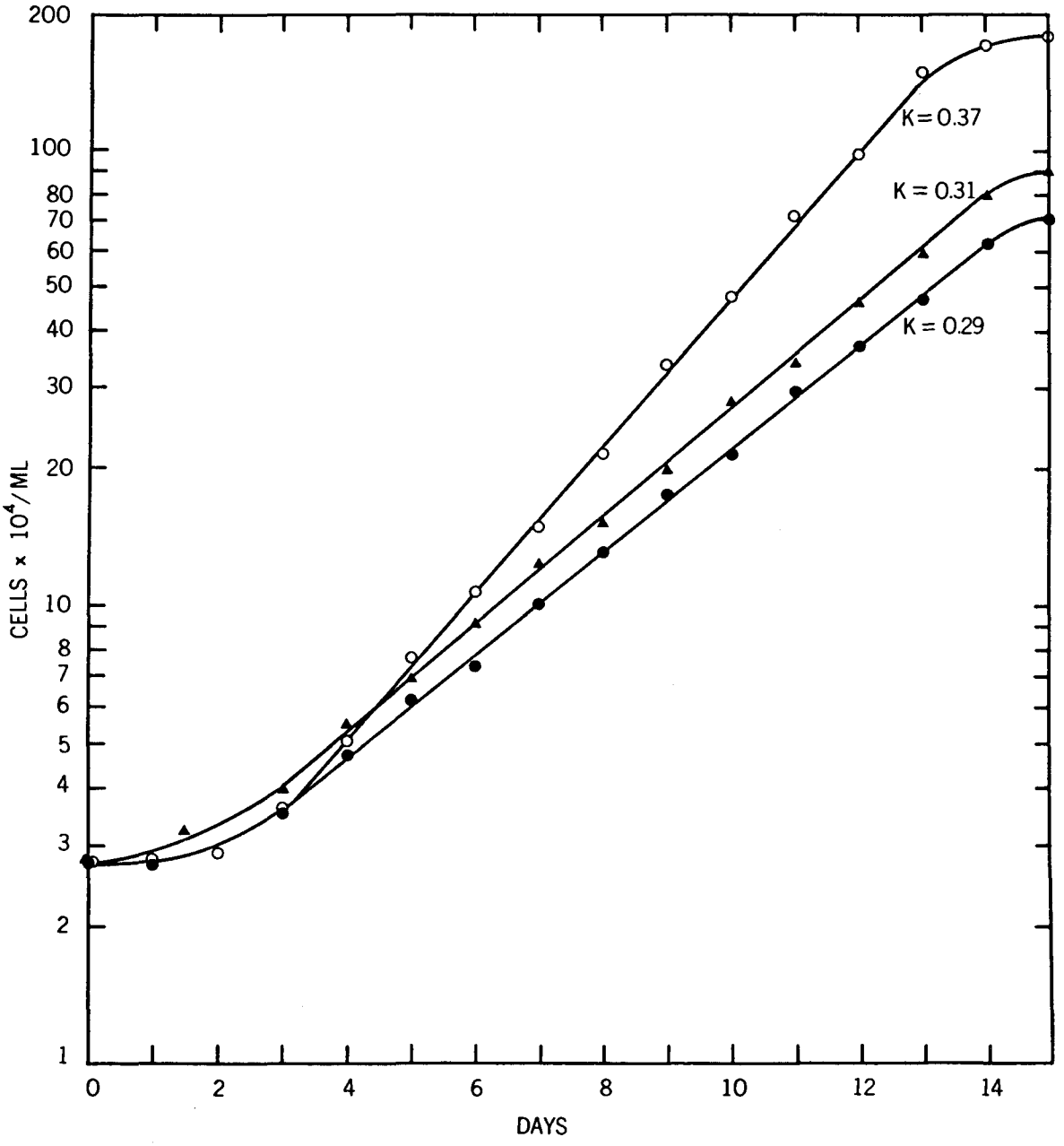

200
100
80
70
60
50
40
30
20
10
8
7
6
5
4
3
2
1
CELLS x 10^4/ML
0
2
4
6
8
10
12
14
DAYS
K=0.37
K = 0.31
K = 0.29

Figure 1.4 Growth of Dunaliella tertiolecta in blue (○), green (●) or white light (△). Light intensity 7.9×10^3 ergs cm^{-2} sec^{-1}.

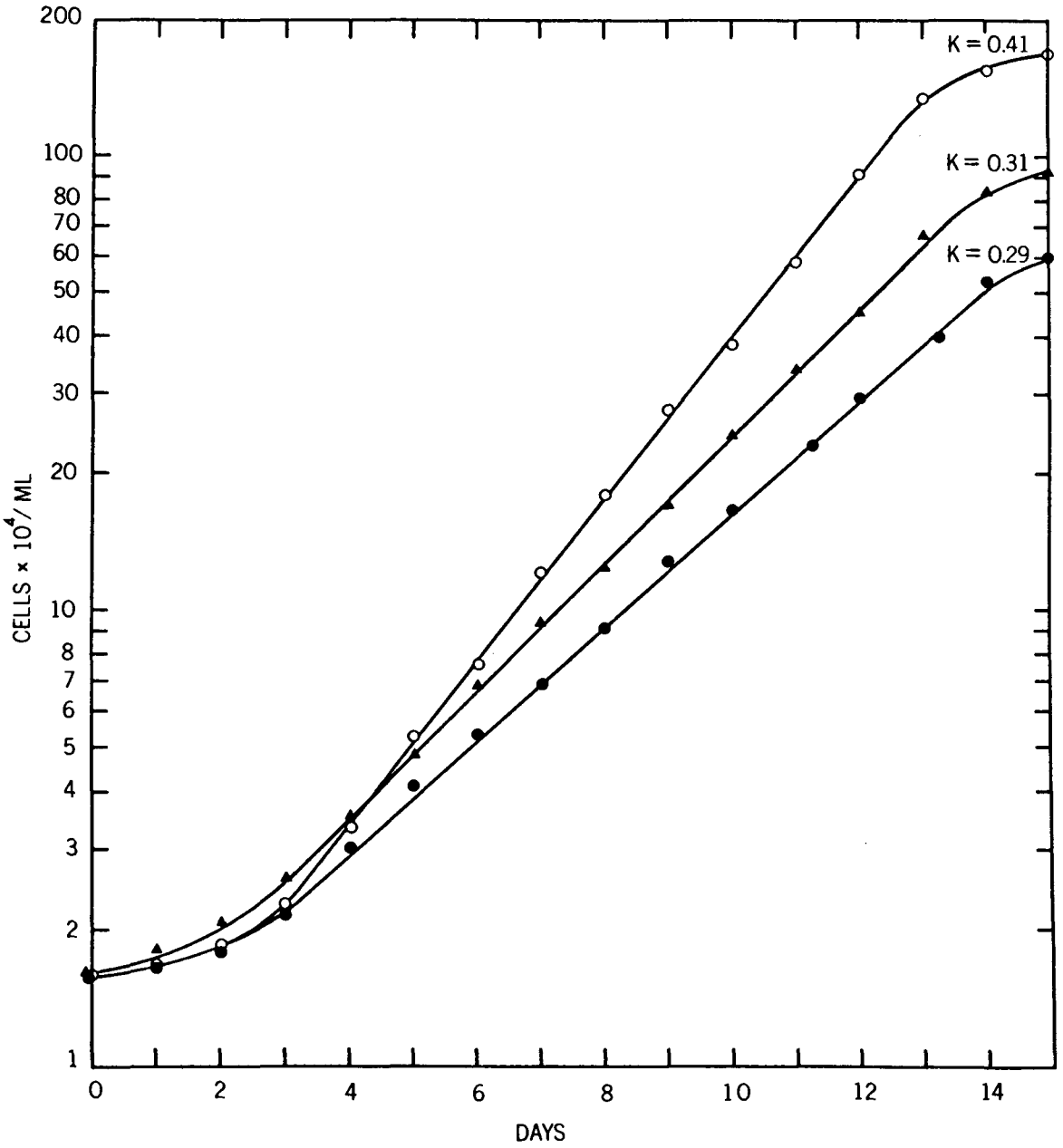

200
100
80
70
60
50
40
30
20
10
8
7
6
5
4
3
2
1
CELLS × 10⁴/ML
0
2
4
6
8
10
12
14
DAYS
K = 0.41
K = 0.31
K = 0.29

Table 1.4

Distribution of ^{14}C among the ethanol-soluble and ethanol-insoluble fractions in Cyclotella nana and Dunaliella tertiolecta. The cells were exposed to $^{14}CO_2$ for 30 minutes at or near saturation intensity or low intensity (roughly 10% of saturating intensity), under blue, green or white light.

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				
Cyclotella	white	12.8	32.7	67.3
	blue	22.4	64.1	35.9
	green	11.0	60.2	39.8
Dunaliella	white	19.0	10.1	89.9
	blue	24.6	71.2	28.8
	green	12.4	66.8	33.2
Low intensity				
Cyclotella	white	3.5	33.4	66.6
	blue	6.2	61.4	38.6
	green	3.2	59.1	40.9
Dunaliella	white	5.9	13.8	86.2
	blue	7.4	72.5	27.5
	green	2.6	69.3	30.7

ethanol-insoluble fraction compared with 10 - 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 1.4). In white light, the ethanol-soluble fraction consistently contained 85 - 90% of the total ^{14}C assimilated in Dunaliella and 65 - 70% of the ^{14}C assimilated in Cyclotella. These species differences were not apparent in blue or green light.

Hydrolysis of the ethanol-insoluble fraction showed that protein amino acids accounted for about 96% of the ^{14}C fixed in this fraction in blue or green light (Table 1.5). 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 1.6. The main soluble carbohydrate in Dunaliella is glycerol. Glucose and trace quantities of sucrose also occurred. Glucose was the primary soluble carbohydrate in Cyclotella. Trace quantities of fructose, ribose and mannose and one unknown consistently appeared.

When Cyclotella and Dunaliella were exposed to blue light, there was an increase in the radioactivity of aspartic acid,

Table 1.5

Total $^{14}C/10^9$ cells in the ethanol-insoluble fraction and in the products resulting from hydrolysis of this fraction. The cells were exposed to $^{14}CO_2$ for 30 minutes under high intensities of blue, green or white light.

Organism	Light quality	ethanol-insoluble fraction	Protein-amino acids		Carbohydrates		Unrecovered
		μCi	μCi	%*	μCi	%*	%
Cyclotella	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	--
Dunaliella	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

* % of total ^{14}C fixed in the ethanol-insoluble fraction

TABLE 1.6

Distribution of ^{14}C among compounds of the ethanol-soluble fraction in *Cyclotella* and *Dunaliella*. These cells were exposed to $^{14}CO_2$ for 30 minutes in blue, green or white light at saturating or high intensities. Cell number approximately (a) 3×10^5 cells/ml, (b) 1×10^5 cells/ml.

	% of total ^{14}C fixed in the ethanol-soluble fraction											
	A						B					
Organism	Cyclotella			Dunaliella			Cyclotella			Dunaliella		
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	19.3

– below detectable limit

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. Dunaliella did differ in that no radioactivity was detected in glutamine after 30 min of photosynthesis. The radioactivity in glycerol, the primary carbohydrate, decreased in blue light compared with white light.

When Cyclotella and Dunaliella were incubated in green light, the quantity of serine, aspartic, glutamic, malic, and fumaric acids increased relative to white light, but the concentration of glucose in Cyclotella, glycerol in Dunaliella, the phosphate esters and glycine decreased.

A series of experiments were carried out at light intensities approximately 10% of those used in the initial experiments. The results with Cyclotella and Dunaliella were similar to those obtained when the cells were grown at saturating or high intensities of blue, green, or white light (Table 1.7). The data in Tables 1.6 and 1.7 indicate that the effect of light quality on the nature of the photosynthate is independent of the photosynthetic rate. Also, cell concentration apparently did not have a very great influence on the distribution of compounds in the ethanol-soluble fraction.

TABLE 1.7

Distribution of ^{14}C among compounds of the ethanol-soluble fraction of *Cyclotella* or *Dunaliella*. These cells were exposed to $^{14}CO_2$ for 30 minutes in blue, green or white light at light intensities approximately 10% of the intensities of previous experiments. Cell numbers approximately (a) 3×10^5 cells/ml, (b) 1×10^3 cells/ml.

	a						b					
Organism	Cyclotella			Dunaliella			Cyclotella			Dunaliella		
	% of total ^{14}C fixed in the ethanol-soluble fraction						% of total ^{14}C fixed in the ethanol soluble fraction					
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	.9	2.8	1.2	–	1.0	.8	2.2	3.1	3.4	1.0	2.1	1.4
Glutamine	.6	1.5	.9	–	–	–	.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	.9	.7	.9	1.0	1.0	–	1.1	1.0	1.1	.5	–	.9
Glucose	16.6	8.6	9.3	2.1	1.1	–	15.6	12.1	12.6	1.8	.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

– below detectable limits

DISCUSSION

Growth, photosynthetic rate and the nature of the photosynthate of planktonic algae changes in response to variations in a number of environmental factors such as temperature, CO_2 concentration, salinity, and light intensity. The results reported here for Cyclotella nana and Dunaliella tertiolecta (Fig. 1.2, Tables 1.1 - 1.3) indicate a response to the spectral quality of available light.

The effects of light quality on light intensity-photosynthesis curves were considered (Fig. 1.2A, 1.2B). 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 over-all magnitude of photosynthesis the curves have been normalized and I_k values, which represent the ratio between the rate of the photochemical processes in photosynthesis per unit energy and the maximum rate of enzymatic processes, have been determined (Talling 1957). 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. The higher I_k and light saturated rate of photosynthesis in blue light (Fig. 1.2A, 1.2B) suggests that concentrations of photosynthetic enzymes are probably higher in blue light grown cells. Furthermore, the slope of the linear

portion of the curves in Fig. 1.2A suggests that Cyclotella 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. 1.2A are normalized (Fig. 1.2B) 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. Low temperature, CO_2, nutrient concentrations and light intensity all affect dark reactions to a greater degree than light reactions (Yentsch and Lee 1966, Steemann Nielsen and Hansen 1961, Rabinowitch 1951). Yentsch and Lee (1966) also suggested that lower I_k values, such as observed when Cyclotella 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 Jorgensen 1968) is occurring. The results reported here indicate that algae adapt to light quality although the extent of adaptation is not sufficient to equalize the quantum efficiency of photosynthesis in the three light systems.

The relative respiration rates in Cyclotella and Dunaliella,

estimated by the method of Steemann Nielsen and Hansen (1959b) were lower in blue light cells than in those grown in green or white light (Fig. 1.2A). 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 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 Cyclotella and Dunaliella 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 Fig. 1.3 and 1.4.

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 1.3) was similar to the blue:white ratio (Table 1.2) suggesting that the blue light is overriding 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). The data presented in this section suggest a low carotenoid efficiency in cells exposed to a mixture of blue and green light. The mechanism involved is uncertain. However, this type of response is not new. Hauschild et al. (1962a) observed that in photosynthesizing Chlorella 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 and Dunaliella grown in blue or green light compared with white light.
(1) In blue or green light 60 - 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 - 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 1.5. A minimum of 92% of the activity was recovered on hydrolysis of proteins and a further 1 - 7% recovered as sugars. Of the 16 - 18 common protein amino acids, aspartic acid, glutamic acid and alanine contained most ^{14}C activity (Table 1.8). Our results agree with those 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 - 580 nm)) changes the rate of protein synthesis but not its composition. In contrast, Hauschild et al. (1962a, 1962b) were unable to demonstrate any differential effect of blue light on the incorporation of ^{14}C into the protein of several freshwater algae. However, blue light stimulated an increased proportion of $^{14}CO_2$ into the protein component of bean and tobacco leaves (Voskresenskaya 1956).

Glucose, probably derived from the hydrolysis of starch, was the most significant radioactive sugar recovered from Dunaliella on carbohydrate hydolysis. Glucose and small quantities of mannose were recovered from Cyclotella. These two sugars may have been derived from a substance similar to

Table 1.8

Distribution of ^{14}C among the protein amino acids after 30 min of photosynthesis by Cyclotella and Dunaliella in blue, green or white light at saturating or high intensities. Approximately 3×10^5 cells/ml.

% of total ^{14}C fixed in the protein fraction						
	Cyclotella			Dunaliella		
	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

laminarin, a reserve polysaccharide that 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 saturating intensities and at low intensities. Apparently the changes in distribution of ^{14}C among the photosynthetic products are not dependent on 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, glycine and probably glycolic acid. No conclusions were drawn from measurements of radioactivity in glycolic acid since this compound is highly volatile. Laboratory studies indicated that a large proportion of it was lost between the killing procedure and the final measurements.

If the concentration of free amino acids is expressed as a percent of the total ^{14}C fixed by the cells (Table 1.9), 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

Table 1.9

The distribution of ^{14}C among compounds of Cyclotella or Dunaliella as a percentage of the total ^{14}C fixed after 30 min photosynthesis in blue, green or white light at saturating or high intensities.

Organism	Cyclotella			Dunaliella		
	% of total ^{14}C fixed					
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
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

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 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. The data do not identify the reactions involved in CO_2 fixation. They do however, show that short wavelength light switches the direction of algal metabolism in favor of

protein synthesis in the two algae Cyclotella nana and Dunaliella tertiolecta while white light promotes the formation of carbohydrates.

The results obtained in this investigation lend support to some earlier work that alterations in the spectral composition of light is an important factor determining the plant's response to environmental conditions. Light quality alters the pathway of synthesis of carbon leading to differences in the qualitative and quantitative relationships between the direct products of photosynthesis. The data suggest that both the CO_2 fixation and the distribution of the photosynthetic metabolites 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.

Chapter 2

PROTEIN, RNA, DNA AND PHOTOSYNTHETIC PIGMENTS IN TWO SPECIES OF MARINE PLANKTON ALGAE DURING GROWTH IN LIGHT OF DIFFERENT SPECTRAL QUALITY

INTRODUCTION

In the previous chapter evidence was presented that light of different spectral quality, but of the same intensity, produces differences in cell growth rates, CO_2 fixation rates, and photosynthetic products in two species of marine plankton algae, Cyclotella nana Hustedt and Dunaliella tertiolecta Butcher. Rates of increase in cell numbers were highest in blue light, intermediate in white and lowest in green light. Responses to light quality which resulted in an increase in the protein concentration in Cyclotella and Dunaliella grown in blue and green light relative to that in white light were also described. The observed differences in division rates should reflect differences in rates of DNA synthesis since the formation of daughter cells is closely related to a preceding increase in DNA (Ruppel 1962). Any increase in rates of protein synthesis should be closely related to an increase in RNA (Ruppel 1962). Therefore, changes in RNA and DNA concentrations may be expected in Cyclotella and Dunaliella when grown in light of different spectral quality.

Differences in light quality also appear to influence the concentration of the various photosynthetic pigments. Observations in Chapter 1 suggest that pigment concentrations are higher in blue and lower in green than in white light. The work reported here describes a series of experiments designed to investigate the effect of variations in light

quality on the rate of synthesis of protein, RNA, DNA and photosynthetic pigments.

MATERIALS AND METHODS

Axenic cultures of two species of marine plankton algae were used in this investigation, the diatom Cyclotella nana Hustedt and the green flagellate Dunaliella tertiolecta Butcher. The culture medium and methods of culture were the same as described in Chapter 1. The concentration of chlorophyll a and b or c and the total carotenoids was measured daily for 10 - 15 days in cultures grown under blue, green or white light of the same intensity. Pigments were extracted in 90% acetone in the manner described by Strickland and Parsons (1965) with the exception that 1 drop of 0.5% dimethylamine instead of $MgCO_3$ was added to the extract. A sonic disruptor (Bronwill Biosonic, 125 watts) was used to facilitate pigment extraction. Three 30-sec periods of sonification at 70% maximum power were sufficient to ensure cell disruption and maximum extraction of pigments. The use of the sonic disruptor increased measured pigment concentrations 18 - 30% over those obtained without treatment. Optical densities of the acetone extracts were measured in 1-cm cuvettes with a Cary Model 14 Recording Spectrophotometer. Pigment concentrations were calculated using the trichromatic equations of Strickland and Parsons (1965).

Pigments were extracted from cultures grown for 9 days under blue, green or white light to examine possible changes in the concentrations of β-carotene and the various xanthophylls in the carotenoid fraction. The pigments were separated by two-dimensional paper chromatography. To prepare the pigments for chromatography, each acetone extract was mixed in a separatory funnel with an equal volume of diethyl ether and washed thoroughly with 10% (V/V) NaCl. Chromatograms were run in the dark in chromatographic tanks containing a beaker of silica gel to keep solvents and atmosphere dry. The solvent system for the first direction consisted of fresh 4% (V/V) propanol in redistilled hexane. The second solvent system consisted of 30% (V/V) chloroform in redistilled hexane. The chlorophylls were eluted from the chromatograms with acetone, the carotenoids with ethyl ether. The optical density of each pigment fraction was determined. Pigments were identified after elution from the chromatograms by the absorption spectra described by Goodwin (1955) and Smith and Benitez (1955), although the absorption spectra described by Jeffrey (1963) for chlorophyll c and fucoxanthin (Parsons and Strickland 1963) were also used. The R_f values reported by Jeffrey (1961) were also used for identification. The extinction coefficients used in calculating the pigment concentrations were those given by Jeffrey (1961) except for chlorophyll c (Jeffrey 1963) and fucoxanthin (Parsons and Strickland 1963).

where the extinction coefficients were unknown (the minor xanthophylls) the extinction coefficient for β-carotene was also used (Jeffrey 1961).

In another series of experiments the protein, RNA and DNA concentrations in cells grown under blue, green or white light were measured daily for 10 - 15 days after new cultures were established. New cultures were started under appropriate light conditions from stock cultures kept in white light.

DNA was measured using the fluorometric method described by Holm-Hansen et al. (1968). Aliquots of the culture were filtered through Millipore HA filters (25 mm, 0.45$_\mu$ pore diam.), the cells and filter then suspended in 5 ml of absolute acetone and broken down with a sonic disruptor. The preparation was then centrifuged and the supernatant discarded. Substances which might interfere with the DNA assay were then extracted from the sediment in the following sequence: a) four times with absolute acetone; b) once with 90% acetone; c) once with cold (5 C) 10% TCA; d) twice with 95% ethanol. After drying, diaminobenzoic acid solution (DABA.2HCl) was added to the sediment and the mixture heated for 1 hr. Then, 0.6 N perchloric acid was added and the fluorescence of the DNA-DABA complex was measured with a Baird Atomic Fluorescence Spectrophotometer. The concentration of DNA in the sample was determined from a standard calibration curve prepared with calf thymus DNA.

Ribonucleic acid concentrations were determined with the orcinol method of Schneider (1957). Aliquots of the culture were filtered and impurities extracted as described above for DNA. The sediment was washed with 95% ethanol and the supernatant discarded. Then 1.3 ml of 10% TCA and 1.3 ml of distilled water were added to each sample and the mixture was heated at 90 C for 15 min. After centrifuging 1.5 ml of the supernatant fluid was added to 1.0 ml of distilled water and 1.5 ml of orcinol reagent. The mixture was then heated in a water bath at 90 C for 30 min. The solution was cooled and its optical density measured at 660 nm. Ribose dissolved in distilled water was used for calibration.

Protein was measured with the phenol reagent of Folin and Ciocalteu (Lowry et al. 1951). The plant cells were disrupted sonically and solubilized in a copper alkali solution for 1 hr at 100 C. The colour developed on addition of the phenol reagent was measured at 750 nm. The method was standardized with bovine serum albumin.

RESULTS

Pigment concentrations from cultures grown for 9 days under each light regime and determined chromatographically are presented in Table 2.1. Pigment concentrations measured 12 - 20%

Table 2.1

Chlorophyll and carotenoid concentrations in *Cyclotella nana* and *Dunaliella tertiolecta* after 9 days growth under blue, green or white light. Light intensity was 7.9×10^3 ergs cm^{-2} sec^{-1}. Each value represents the mean of six samples. Cell size was the same under the three light regimes. Pigments were separated by paper chromatography.

	white		blue		green	
	$\mu g/10^6$ cells	% total carotenoids	$\mu g/10^6$ cells	% total carotenoids	$\mu g/10^6$ cells	% total carotenoids
Dunaliella						
chlorophyll *a*	2.68		2.98		2.36	
chlorophyll *b*	1.34		1.69		1.14	
β-carotene	0.32	26.7	0.36	31.1	0.44	33.3
neoxanthine	0.15	12.4	0.08	11.9	0.17	12.7
lutein	0.47	39.7	0.39	42.6	0.58	43.6
violaxanthin	0.25	21.2	0.13	14.4	0.14	10.4
total carotenoids	1.19		0.96		1.33	
chlorophyll *b*:*a*	0.50		0.57		0.48	
carotenoids:chlorophyll *a*	0.45		0.32		0.56	
Cyclotella	$\mu g/10^6$ cells	% total carotenoids	$\mu g/10^6$ cells	% total carotenoids	$\mu g/10^6$ cells	% total carotenoids
chlorophyll *a*	1.23		1.48		1.07	
chlorophyll *c*	0.27		0.34		0.19	
β-carotene	0.04	2.9	0.05	4.7	0.06	3.7
fucoxanthin	0.93	68.4	0.82	76.6	1.16	71.5
neofucoxanthin	0.32	23.5	0.16	14.9	0.35	21.6
diadinoxanthin	0.04	2.7	0.02	1.9	0.03	1.9
diatoxanthin	0.03	2.4	0.02	1.5	0.02	1.9
total carotenoids	1.36		1.07		1.62	
chlorophyll *c*:*a*	0.22		0.23		0.18	
carotenoids:chlorophyll *a*	1.11		0.72		1.60	

higher when determined by chromatography than by spectrophotometry. In Dunaliella the ratio of chlorophyll b:a in cells grown in blue light (0.57) was significantly higher ($p < 0.001$) than in cells grown in white and green light (0.50 and 0.48 respectively). The ratio of chlorophyll c:a in Cyclotella grown in green light (0.18) was lower ($p < 0.001$) than cells grown under white or blue light (0.22 and 0.23 respectively). The carotenoid:chlorophyll a ratios in Cyclotella were 1.60, 1.11 and 0.72 in green, blue and white light respectively.

The absolute and relative concentrations of the individual carotenoids were quite variable in light of different spectral qualities (Table 2.1). The concentration of β-carotene, the major light-absorbing carotenoid in Dunaliella was significantly higher in blue and green than in white light. The β-carotene concentrations in Cyclotella were also greater in blue and green than in white light. The concentration relative to the total carotenoid fraction increased from 2.4% in white light to 4.7% and 3.7% in blue and green light. The concentration in both blue and green light was significantly higher than in white light ($p < 0.001$). On the other hand, the concentration of fucoxanthin, the major light-absorbing carotenoid in Cyclotella, was lower in blue light and higher in green than in white light. The concentration of fucoxanthin as a fraction of the total carotenoids was highest under blue, 76.6%, compared with 71.5% and 68.4% in

green and white light. The concentration in blue light was significantly higher than in green or white light ($p < 0.01$) respectively. The absolute and relative values of the minor xanthophylls were also quite variable. The total concentration of carotenoids in the two algae varied with light quality in the following manner: blue < white < green.

Tables 2.2 and 2.3 show the ratios of chlorophyll a, b, c and carotenoids in Cyclotella and Dunaliella grown in blue, green or white light of the same intensity. These values, which are the ratios of pigment concentrations in blue or green light relative to white light, are means of daily measurements from several different cultures of the two algae grown for periods of 10 - 15 days. The blue:white chlorophyll ratios were greater than 1.0, the green:white ratios less than 1.0. Thus, the chlorophyll concentrations in these two algae vary with light quality in the following manner: blue > white > green.

Differences in the concentration of protein in algae grown under the three light regimes were observed 24 hr after the establishment of new cultures using cells from stock cultures in white light. The protein concentrations and the ratios of protein in blue or green light to white light are presented (Tables 2.4 and 2.5). The concentration of protein in both plankton algae is higher in blue and lower in green than in white light. The blue:white ratio is approximately 1.60 and

Table 2.2

Ratios of the concentrations of chlorophylls *a* and *c* and total carotenoids in *Cyclotella nana* grown in blue or green light relative to those in white light. Light intensity was 7.9×10^3 ergs cm^{-2} sec^{-1}. Each value represents the mean of daily measurements on the same culture over a 10 - 15 day period. Standard deviation for each series is shown.

Ratios relative to white light					
Blue light			Green light		
Chlorophyll *a*	Chlorophyll *c*	Carotenoids	Chlorophyll *a*	Chlorophyll *c*	Carotenoids
1.21 ± 0.09	1.35 ± 0.13	0.65 ± 0.06	0.80 ± 0.03	0.65 ± 0.08	1.14 ± 0.08
1.28 ± 0.11	1.29 ± 0.06	0.63 ± 0.09	0.82 ± 0.06	0.63 ± 0.05	1.19 ± 0.07
1.21 ± 0.10	1.33 ± 0.03	0.71 ± 0.05	0.79 ± 0.02	0.63 ± 0.05	1.23 ± 0.08
1.25 ± 0.08	1.31 ± 0.06	0.68 ± 0.06	0.83 ± 0.05	0.66 ± 0.04	1.21 ± 0.08
1.26 ± 0.09	1.33 ± 0.05	0.70 ± 0.04	0.79 ± 0.05	0.61 ± 0.07	1.17 ± 0.07

Table 2.3

Ratios of the concentrations of chlorophylls *a* and *b* and total carotenoid content in *Dunaliella tertiolecta* grown in blue or green light relative to that in white light. Light intensity 7.9×10^3 ergs cm^{-2} sec^{-1}. Each value represents the mean of daily measurements on the same culture over a 10 - 15 day period. Standard deviation for each series is shown.

Ratios relative to white light					
Blue light			Green light		
Chlorophyll *a*	Chlorophyll *b*	Carotenoids	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
1.11 ± 0.08	1.34 ± 0.05	0.83 ± 0.06	0.88 ± 0.05	0.88 ± 0.06	1.09 ± 0.06
1.17 ± 0.05	1.33 ± 0.04	0.81 ± 0.09	0.75 ± 0.05	0.83 ± 0.05	1.13 ± 0.05
1.11 ± 0.05	1.29 ± 0.07	0.82 ± 0.06	0.69 ± 0.06	0.86 ± 0.05	1.17 ± 0.05
1.13 ± 0.04	1.30 ± 0.07	0.86 ± 0.04	0.76 ± 0.07	0.84 ± 0.04	1.15 ± 0.06
1.14 ± 0.05	1.31 ± 0.06	0.85 ± 0.04	0.78 ± 0.04	0.82 ± 0.03	1.19 ± 0.04

Table 2.4

Concentrations of protein, RNA and DNA in Cylotella nana grown in blue, green or white light. The ratios of protein, DNA and RNA in blue or green light relative to those in white light are also presented. Light intensity was 7.9×10^3 ergs cm^{-2} sec^{-1}. Each value represents the mean of daily measurements on same culture over a period of 10 - 15 days. Standard deviations for each series are shown.

	μg protein/10^6 cells		ratios of protein concentrations	
white	blue	green	blue:white	green:white
10.1±0.1	16.9±0.2	8.9±0.1	1.68	0.89
9.3±0.5	14.9±0.3	7.5±0.2	1.60	0.81
10.3±0.1	16.4±0.1	8.9±0.2	1.59	0.86
9.8±0.3	16.2±0.2	7.7±0.4	1.65	0.79
10.9±0.3	17.5±0.5	9.9±0.3	1.61	0.91
	μg RNA/10^6 cells		ratios of RNA concentrations	
0.91±0.08	1.14±0.03	0.79±0.03	1.25	0.87
0.98±0.02	1.23±0.07	0.81±0.02	1.26	0.83
0.83±0.03	1.15±0.08	0.76±0.02	1.38	0.92
0.96±0.05	1.25±0.02	0.78±0.02	1.81	0.81
0.85±0.05	1.10±0.04	0.73±0.03	1.29	0.86
	μg DNA/10^7 cells		ratios of DNA concentrations	
0.88±0.02	0.99±0.01	0.79±0.02	1.13	0.90
0.91±0.01	0.99±0.03	0.83±0.04	1.09	0.92
0.92±0.06	1.02±0.03	0.82±0.02	1.11	0.90
0.86±0.03	0.93±0.02	0.74±0.03	1.08	0.86
0.86±0.02	1.00±0.03	0.76±0.01	1.16	0.88

Table 2.5

Concentration of protein, RNA and DNA in Dunaliella tertiolecta grown in blue, green or white light. The ratios of protein, RNA and DNA in blue or green light relative to those in white light are also presented. Light intensity was 7.9×10^3 ergs cm^{-2} sec^{-1}. Each value represents the mean of daily measurements on the same cultures over a period of 10 - 15 days. Standard deviations for each series are shown.

μg protein/10^6 cells			ratios of protein concentrations	
white	blue	green	blue:white	green:white
78.2±3.2	118.1±2.1	72.7±1.1	1.51	0.93
81.3±1.5	129.3±4.0	77.6±2.3	1.59	0.85
75.5±1.9	123.1±2.0	64.9±2.9	1.63	0.86
80.9±2.8	127.8±2.2	72.0±1.8	1.58	0.89
76.4±1.6	124.5±3.6	66.5±2.0	1.63	0.87
μg RNA/10^6 cells			ratios of RNA concentrations	
14.1±0.7	17.1±2.1	11.7±1.1	1.21	0.83
14.9±0.9	17.7±1.0	13.6±1.1	1.19	0.91
12.3±1.0	15.7±1.2	10.8±1.1	1.28	0.88
15.9±0.9	20.5±1.8	13.5±1.0	1.29	0.85
12.1±1.2	15.2±1.9	10.0±1.6	1.26	0.83
μg DNA/10^6 cells			ratios of DNA concentrations	
0.90±0.04	0.97±0.02	0.82±0.02	1.08	0.91
0.88±0.01	0.98±0.04	0.82±0.03	1.11	0.93
0.95±0.03	1.04±0.05	0.84±0.01	1.09	0.88
0.93±0.03	0.98±0.02	0.84±0.04	1.05	0.90
0.87±0.02	0.97±0.02	0.79±0.01	1.12	0.91

the green:white ratio 0.90.

Changes in RNA and DNA concentrations under light of different spectral quality corresponded to the protein changes. The blue:white light RNA ratios were approximately 1.3 (Tables 2.4 and 2.5). The comparable green:white ratios were approximately 0.90. The blue:white and green:white ratio for DNA were approximately 1.10 and 0.90 respectively (Tables 2.4 and 2.5).

DISCUSSION

In the previous chapter data was presented which suggested that a relatively high rate of light-saturated photosynthesis observed when Cyclotella was grown in blue compared with green or white light was attributable, in part at least, to a higher concentration of photosynthetic pigments. These differences in pigment concentrations are verified by the data presented here. The ratios of chlorophyll a concentrations in blue or green to white light of the same intensity show that chlorophyll a was highest when cells were grown in blue light and lowest in green light (Tables 2.2 and 2.3). The chlorophyll b concentrations in Dunaliella and chlorophyll c concentrations in Cyclotella varied in a similar manner relative to light quality. Carotenoid concentrations in the two plankton algae were highest

in green and lowest in blue light. However, the total concentrations of all pigments were highest in blue and lowest in green light.

Responses of this type are frequently discussed in terms of Englemann's theory of "complementary chromatic adaptation". This suggests that light of a limited spectral region results in an increase in the concentration of those pigments which strongly absorb the incident radiation and a decrease in the concentration of pigments which weakly absorb the radiation. Cyclotella and Dunaliella grown in blue light, which is strongly absorbed by chlorophylls and weakly absorbed by carotenoids, had a higher chlorophyll and lower carotenoid concentration than cells grown in white light. Green light, which is strongly absorbed by carotenoids and weakly absorbed by chlorophylls, was associated with a higher concentration of carotenoids and a lower concentration of chlorophyll.

While the total concentration of carotenoids was highest in green and lowest in blue light, this was not a consistent trend exhibited by all the carotenoids (Table 2.1). For example, there was less violaxanthin in Dunaliella in blue and green than in white light. Further, both algal species contained more β-carotene in blue and green than in white light. The reasons for these individual variations in the carotenoids in various light qualities is not clear.

Since the intensities of blue, green and white light were the same, it is clear that the observed pigment concentrations were due to a response to light quality. Similar results have been observed by other investigators. Fujita and Hattori (1962) reported that changes in chlorophyll a and phycobilin concentrations in Tolypothrix tenuis were the result of light quality rather than intensity. Jones and Myers (1965) reached a similar conclusion from work with Anacystis nidulans. Brody and Emerson (1959) reported that chlorophyll a and phycoerythrin concentrations changed in response to light quality at low light intensities and to light intensity when incident radiation was high. Pigment concentrations were not measured at low intensities. However, light intensity-photosynthesis curves presented in Chapter 1 (Fig. 1.2) suggest that differences in pigment concentrations also existed when cells were exposed to equal, low intensities of blue, green or white light.

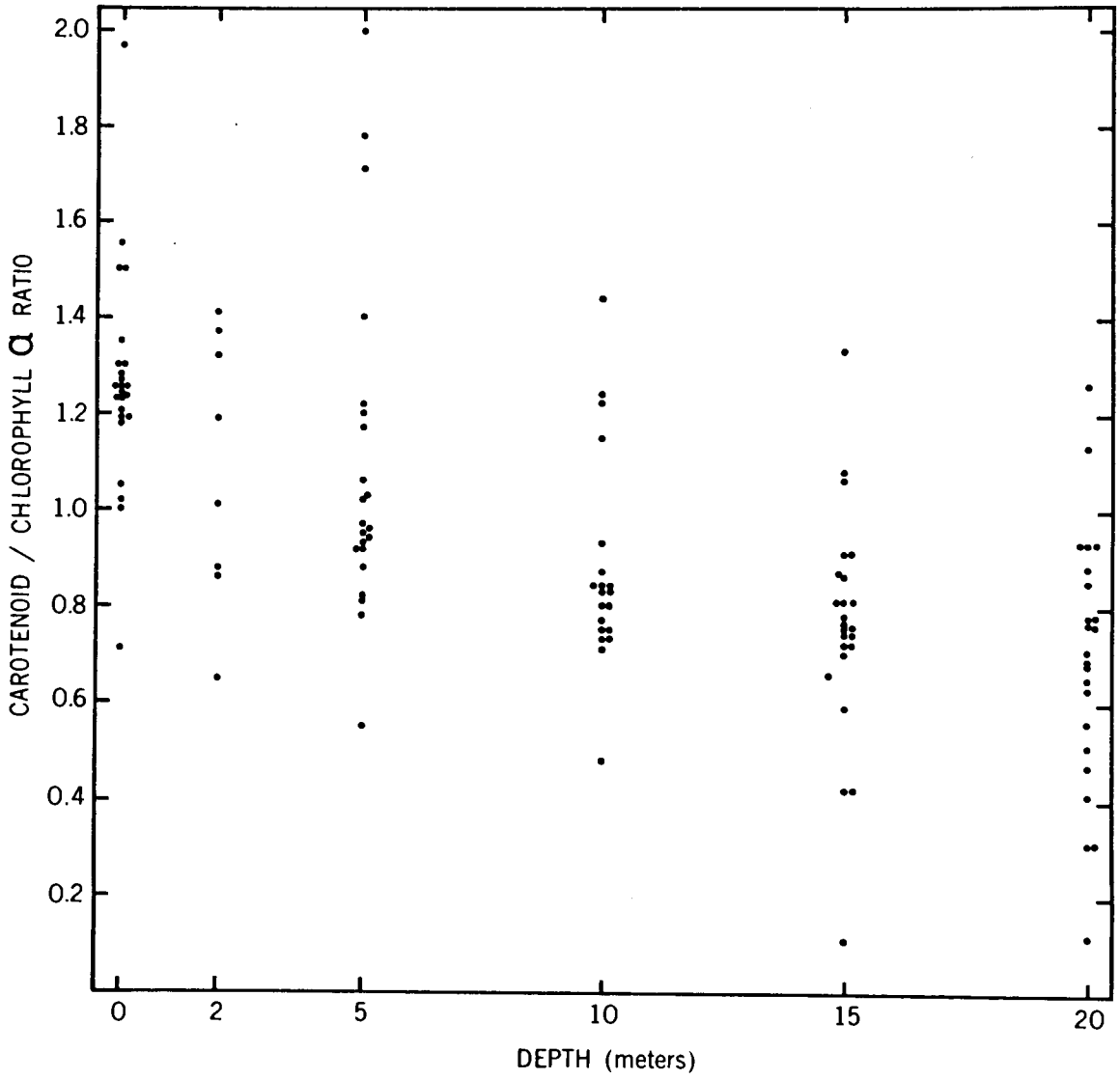
The possibility of adaptation of plankton algae to the spectral quality of light has received little attention, although a number of investigators have examined adaptation to light intensity (Steemann Nielsen and Jorgensen 1968, Jorgensen and Steemann Nielsen 1965; Saijo and Ichimura 1962; Ryther and Menzel 1958, Steemann Nielsen and Hansen 1961). These studies revealed differences in chlorophyll concentrations and light saturated rates of photosynthesis in populations of plankton

algae according to the light intensities under which they are growing. In general, algae found at the surface, where intensities are high, had a higher maximum photosynthetic rate and lower chlorophyll concentration per cell than algae in the lower photic zone. Steemann Nielsen and Hansen (1961) however, indicated that other factors, such as temperature influence these adaptive responses of algae. The results presented in this chapter would suggest that the responses of populations of plankton algae to light observed by these investigators were due, at least in part, to the spectral composition of radiation.

In this investigation pigment concentrations in natural populations were not measured. However, laboratory data have been compared with those from a field study in Saanich Inlet (Fulton et al. 1969). The carotenoid:chlorophyll a ratios calculated from the Saanich Inlet data from May to July 1968 are shown in Fig. 2.1. The carotenoid:chlorophyll a data show considerable scatter. The estimated median values at each depth are used in this discussion.

The Saanich Inlet populations consisted mainly of the diatoms Skeletonema, Thalassiosira and Chaetoceros, at times, small flagellates and occasionally Rhizoselenia. At the surface, the carotenoid:chlorophyll a ratio of 1.23 corresponds to the carotenoid:chlorophyll a ratio of 1.11 for Cyclotella

Figure 2.1 Ratios of carotenoid:chlorophyll *a* concentrations in phytoplankton populations from various depths in Saanich Inlet, Vancouver Island, B. C. The ratios were calculated from data collected at Saanich Inlet from May to mid July, 1968 (Fulton *et al*. 1969).


CAROTENOID / CHLOROPHYLL a RATIO
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
2
5
10
15
20
DEPTH (meters)

grown in white light (Table 2.1) which is similar to the ratios observed by Jeffrey (1961) for diatoms grown under white light. The median carotenoid:chlorophyll a ratio, which decreased with depth, was 0.87 at 5 m, 0.79 at 10 m and 0.77 at 15 m. The ratios at 10 and 15 m are comparable to the ratio of 0.72 for Cyclotella grown in blue light (Table 2.1). The carotenoid:chlorophyll a ratio in green light was 1.60. At 10 m approximately 1% of the surface light is present. This light is primarily in the blue-green part of the spectrum (T. R. Parsons, personal communication).

Differences in a number of environmental parameters, such as the availability of nutrients (particularly nitrates), temperature, light intensity and light quality, may influence the pigment concentrations in phytoplankton. However, with the exception of light intensity and light quality, these parameters did not vary greatly in the photic zone of Saanich Inlet (Fulton et al. 1969). The contribution of light quality to their results is not clear. However, there is a rough similarity between the results in this investigation and those from the Saanich Inlet populations. The carotenoid:chlorophyll a ratio decreased with depth from a surface value which corresponded to the value for Cyclotella cultures grown in white light to 10 m where the ratios were comparable to those in cultures grown in blue light but substantially lower than those cultured in green light. The

response at 5 m appears to be intermediate to the surface and deep responses. The adaptation of the natural populations may be in response to the blue light rather than to green light. Support for this statement is provided from earlier experiments reported in Chapter 1 (Table 1.3). The photosynthetic response of Dunaliella and Cyclotella grown in blue-green light, similar in distribution to that in the lower photic zone, was the same as the response in blue light at the same intensity. It appears that the observed changes in phytoplankton pigments is, at least in part, a function of the spectral quality of light at various depths in the photic zone.

Results also show that light of different spectral composition has a marked effect on the concentration of proteins, RNA and DNA in Cyclotella and Dunaliella. The protein concentration in both algae was highest in cells grown in blue and lowest in green light (Tables 2.4 and 2.5). Kowallik (1965) and Pirson and Kowallik (1964) reported that the concentration of protein in Chlorella was higher in blue than in white light.

Data in the previous chapter showed that greater percentages of ^{14}C were incorporated into the protein fraction during 30 min of photosynthesis in blue and green than in white light. The blue:white and green:white ratios of ^{14}C in the protein fraction were 3.6 and 1.7 respectively for Cyclotella, 9.8 and 4.6 respectively for Dunaliella. These ratios are considerably

higher than comparable ratios in Tables 2.4 and 2.5. Rapid turnover of newly formed protein may account for these differences. This may produce amino acid pools which are separate from the pools of newly synthesized amino acids destined for protein synthesis. Amino acids derived from protein breakdown have been shown to be isolated within cells from the amino acids destined for protein synthesis. Evidence supporting this concept has been summarized by Bidwell et al. (1964). The amino acids in the inactive pool (with respect to protein synthesis), would then be available for use in other metabolic pathways leading to the formation of amides or other organic compounds. Nitrogen released in the synthesis of other organic compounds might be available for re-utilization in the formation of new amino acids.

Some of the additional protein formed in blue light may be deposited in the cytoplasm or used for the synthesis of enzymes. There is also a possibility that the blue-light enhancement of chlorophyll formation may be due to an accelerated production of certain of the chloroplast proteins which are closely associated with chlorophyll formation.

The RNA concentrations were highest in blue and lowest in green light, corresponding to the differences observed in the protein concentrations (Tables 2.4, 2.5). Pirson and Kowallik (1964) reported a similar increase of RNA in Chlorella grown

under blue light. An increase of RNA in blue light has also been observed in ferns and higher plants (Raghavan 1968, Voskresenskaya and Nechaeva 1967).

The different changes in DNA concentration associated with light quality were similar to those observed for protein and RNA and corresponded to the rates of cell division in the two plankton algae which were highest in blue light and lowest in green light (Chapter 1). Voskresenskaya and Nechaeva (1967) observed a similar effect of blue, green and white light on DNA in barley leaves. Sokawa and Hase (1967) have suggested that the direct effect of light is exerted on DNA synthesis and that cellular division is affected indirectly.

The above results clearly indicate that light of different spectral quality induces changes in concentration of pigments, protein and nucleic acids in these plankton algae. Our results do not prove conclusively that pigment synthesis and other metabolic processes are linked. However, since these changes are associated with differences in light quality it is possible to speculate that both pigment synthesis and other metabolic processes are so interdependent that complementary changes in both systems compensate, to a degree, for environmental alterations. Alternatively, the changes in metabolic products may be due to the absorption of light by different light sensitive pigments such as a flavin (Voskresenskaya and Nechaeva 1967)

or phytochrome (Krotkov 1964). The results that have been observed in the laboratory studies may have their counterpart in natural populations. This, combined with any vertical stability of the water in the photic zone and the consequent inhibition of vertical mixing may result in the development of two or more distinct phytoplankton populations between the surface and the bottom of the photic zone (Steemann Nielsen et al. 1964).

Since the results were reproducible when starting again with fresh cultures from stock cultures kept in white light, the data suggest an environmental adaptation to the specific light quality rather than a genetic change. The similarity of the data in this thesis with those of Fulton et al. (1969) reinforce the suggestion that pigment concentration in natural populations may shift in response to light quality. These changes are associated with complementary changes in the concentration of other metabolic products. Such changes could influence the nature of excreted products. Breakdown of cells in natural populations could also result in different concentrations of organic compounds in different parts of the water column. This might produce vertical differences in the capacity of the water column to support heterotrophic growth (by bacteria, algae and by some invertebrates). The value of phytoplankton as a food source might also vary with depth in the photic zone.

Chapter 3

THE EFFECT OF LIGHT QUALITY ON THE DISTRIBUTION OF ^{14}C IN THE PHOTOSYNTHATE OF PHYTOPLANKTON FROM TWO MARINE INLETS

INTRODUCTION

The culture studies (Chapters 1, 2) have revealed that light of different spectral composition but equivalent intensities alters growth and photosynthetic rates and is associated with differences in concentrations of various photosynthetic pigments, DNA and RNA. However, little is known of the effect of light quality on the chemical composition of natural marine phytoplankton populations.

In this chapter the effect of spectral composition of radiation on the distribution of assimilated ^{14}C and its variation with depth was examined and compared with the results observed in the culture studies. Since light of different wavelengths is selectively attenuated by particulate and dissolved organic and inorganic material in water (Jerlov 1951), vertical differences in the chemical composition of plankton algae might be found within the photic zone. Changes in the nature of metabolic products with depth might affect rates of release of extracellular organic compounds. Shifts in phytoplankton metabolism in the lower photic zone might also change the nutritional value of phytoplankton as a zooplankton food source.

Few studies have been made on the chemical composition of naturally-occurring phytoplankton populations. McAllister et al. (1961) and Antia et al. (1963) observed little change in

the chemical composition of phytoplankton until nutrients were depleted. The concentrations of protein and chlorophyll a then decreased and carbohydrates increased. Olive and Morrison (1967) examined variations in the distribution of ^{14}C in the photosynthetic products of freshwater phytoplankton under natural conditions. They observed a general decrease in the proportion of ^{14}C in the ethanol-soluble fraction with depth and an increase in the ^{14}C incorporated into the protein fraction.

The chemical composition of cultured marine phytoplankton has received some attention. The principal protein amino acids are aspartate, glutamate, alanine and leucine. Glycine, serine, valine, phenylalanine, proline, lysine and occasionally ornithine and histidine also occur (Cowey and Corner 1966, Chau et al. 1967). Up to eighteen different free amino acids have been found in different groups of phytoplankton (Bidwell 1957; Schukerk 1960, Craigie et al. 1966). A wide variety of free carbohydrates occur in phytoplankton. In general, glucose predominates although fructose, sucrose, galactose, mannose, xylose and some sugar alcohols are also found.

The first part of this study was conducted with Saanich Inlet populations during the spring and summer of 1968. The distribution of ^{14}C incorporated into the phytoplankton at various depths was examined. However, under natural conditions

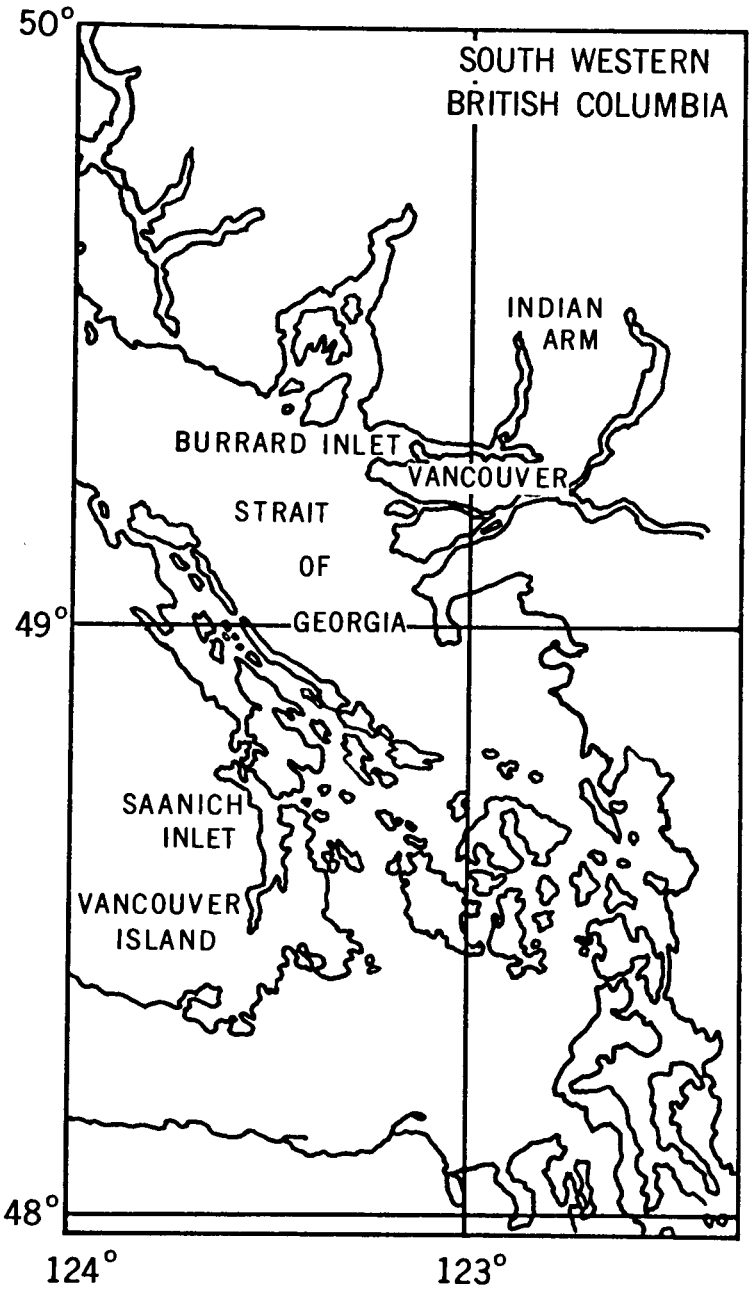
light changes both in quality and intensity with depth. Thus, any differences could be associated with differences in light quality or intensity or a combination of both. Additional studies were conducted in Indian Arm in the spring and summer of 1969 to separate the influence of light quality and intensity on the nature of photosynthetic products in phytoplankton populations.

MATERIALS AND METHODS

The 1968 work was conducted at the field laboratory of the Fisheries Research Board of Canada at Saanich Inlet, a marine inlet at the south-eastern part of Vancouver Island (Fig. 3.1). From May to July temperatures ranged from 8.8 - 20.6 C at the surface and from 7.5 - 14.2 C at 10 m. Salinities ranged from 25.65 - 30.25‰ and 26.16 - 30.76‰ at the surface and 10 m respectively (Fulton et al. 1969). During this period the upper 10 m was only weakly stabilized, and often mixed. Hence, the phytoplankton in the photic zone may have been subjected to considerable vertical transport.

The 1969 study was conducted at Indian Arm, a marine inlet forming part of the Burrard Inlet system of which Vancouver Harbour is part (Fig. 3.1). Salinities ranged from 12.16 - 17.10‰ at the surface while those at 12 m were between 24.91 -

Figure 3.1 Southwestern B. C. showing the locations of the two study areas, Saanich Inlet and Indian Arm.


50°
SOUTH WESTERN
BRITISH COLUMBIA
INDIAN
ARM
BURRARD INLET
VANCOUVER
STRAIT
OF
GEORGIA
49°
SAANICH
INLET
VANCOUVER
ISLAND
48°
124°
123°

27.13‰ (Table 3.2). Temperatures at the surface and 12 m ranged from 7.5 - 14.3 C and 7.2 - 12.4 C respectively. The salinity observations agree with those of Gilmartin (1962) who reported a strong vertical salinity gradient in Indian Arm. A relatively low salinity layer 1.5 - 2.5 m thick overlies a 2 - 5 m transition zone of intermediate salinity and the more saline deeper water. The salinity gradient will restrict vertical circulation. Consequently phytoplankton populations in different parts of the photic zone will probably be exposed to different light regimes for extended periods.

Incident radiation at Saanich Inlet was measured with an Eppley pyrheliometer situated on the roof of the field laboratory about 400 m from the sampling site. Photosynthetically active radiation was assumed to be 50% of that measured by the pyrheliometer (Strickland 1958). A Robitzch-type actinograph was used to measure incident radiation at Indian Arm. Mean surface intensities were reduced by 10% to account for surface loss of radiation by direct reflection and upward scattering (Davis 1941).

Extinction coefficients for Saanich Inlet were calculated from underwater light distribution as measured with a submarine photometer. Both the deck and sea cells, which were fitted with Schott BG-12 blue glass filters, had a maximum response of photocell-filter combination at approximately 432 nm. Cal-

culated extinction coefficients and the data of Jerlov (1951) were used to estimate the attenuation of photosynthetically active light. Subsurface intensities at Indian Arm in the range 380 - 720 nm were calculated from the mean surface intensities and vertical extinction coefficients. Vertical distribution of light was measured with a submarine photometer equipped with blue, green or red filters. Extinction coefficients were calculated in the manner described by Atkins and Poole and applied by Jenkin (1937) and Talling (1957). The spectral distribution of sun and skylight, which was required for calculations, was measured with an ISCO spectroradiometer at noon each sample day. The energy in the blue, green and red regions at different depths was calculated from the extinction coefficients and total available energy. Calculated energies obtained in this way are only approximate although relative values are comparable.

Phytoplankton in Saanich Inlet were sampled at 0.5, 5 and 10 m on May 21, June 11, June 23 and July 16, 1968. Each sample was collected before sunrise in a 5-l PVC water bottle and was divided into two parts. One portion was used to identify the major phytoplankton species and the other was dispensed into a series of light and dark 300-ml BOD bottles into each of which 100 μCi of $^{14}CO_3^{2-}$ were added. The bottles were then suspended at the sampling depth for 10 hr from sunrise.

These procedures avoided any effects of even short exposure of phytoplankton from 5 and 10 m to surface illumination.

Studies in Indian Arm were conducted from late March to mid July, 1969. Phytoplankton were sampled at 0.5 m and 12 m. Approximately 1% of surface radiation remained at 12 m. To avoid any possible effects of exposure of phytoplankton to surface radiation, the method of Watt (1965) was used. To each of a series of light and dark 250-ml boiling flasks 100 μCi of radiocarbon were added after which the flasks were evacuated and lowered to the desired depth on a weighted line. A length of sealed glass tubing extending through the rubber stopper in the mouth of the flask was broken by a sliding messenger. Water was then drawn into the flasks. Half the light bottles incubated at 0.5 m were covered with neutral density filters of 150-mesh monel screen (McAllister et al. 1961). The phytoplankton in these flasks were exposed to intensities approximating those at 12 m. The phytoplankton in the other half of the surface light flasks were exposed to in situ light. The phytoplankton were incubated with radiocarbon from 10 A. M. - 2 P. M.

Following incubation in Saanich Inlet and Indian Arm the bottles or flasks were placed in a dark box and transported to the laboratory where duplicate 10-ml aliquots of each sample were filtered onto separate 25-mm HA Millipore filters. Each filtered sample was placed in 15 ml of scintillator for radio-

carbon assay. Samples were counted for a sufficient length of time to reduce the standard error of counting to 1%. Primary production was calculated according to the method of Strickland and Parsons (1965).

The remainder of each sample was filtered through a 47-mm HA Millipore filter, and the material on the filter dried and frozen. The filtrate was retained for quantitative determination of the labelled organic compounds excreted by the phytoplankton. The algae and the membrane filter were placed in 50 ml of boiling 80% ethanol for 1 hr. The distribution of ^{14}C among various compounds in the ethanol-soluble fraction was determined using the two-dimensional chromatographic and autoradiographic techniques described in Chapter 1. To remove lipids, nucleic acids and polysaccharides, the residues were filtered and extracted successively with: a) 25 ml of redistilled petroleum ether; b) 25 ml 0.2 N cold (1-5 C) perchloric acid for 10 min; c) 25 ml of boiling 10% trichloroacetic acid for 30 min. Extraction periods up to 1 hr were tested for each solvent. However, at least 98% of the total ^{14}C extracted in 1 hr was removed in the times used. The ^{14}C remaining in the residue, referred to as the insoluble fraction, was measured by the wet combustion method of Hofstra (1967).

Dissolved labelled organic carbon in the filtrates was determined by acidifying duplicate samples to pH 3 with 0.1 N

HCl and aerating them vigorously for 1 hr to remove all inorganic carbon. Duplicate 100 µl aliquots of the filtrate were then placed in scintillation fluid for radioassay.

RESULTS

The ^{14}C incorporated into the ethanol-soluble, insoluble and other fractions by Saanich Inlet and Indiam Arm phytoplankton is shown in Tables 3.1 and 3.2 respectively. Generally more than 90% of the total ^{14}C assimilated and retained by the phytoplankton was in the ethanol-soluble and insoluble fractions. The remaining activity was in the petroleum ether, perchloric acid and trichloroacetic acid fractions. The discussion will be confined to a consideration of the occurrence of ^{14}C in the ethanol-soluble and insoluble fractions. Loss of label from the phytoplankton in the form of soluble organic compounds is also considered.

In Saanich Inlet populations the relative activity in the ethanol-soluble fraction generally decreased with depth whereas the relative activity in the insoluble fraction increased with depth. This trend is emphasized if the percent activity in each fraction is presented as a total derived for each depth over the four sets of experiments. The results are as follows:

TABLE 3.1

Distribution of ^{14}C photosynthate in Saanich Inlet phytoplankton sampled and incubated in $^{14}CO_2$ for 10 hr from sunrise at 0.5, 5 and 10m

Date 1968	Depth m	Incident radiation langleys $10hr^{-1}$	Principal cell groups	Photosynthesis $mgCM^{-3}hr^{-1}$	Percentage of ^{14}C recovered in the phytoplankton Soluble in: Petroleum ether %	0.2N PCA %	Boiling 10% TCA %	Ethanol-soluble %	Insoluble residue %	Excreted* organic carbon %
May 21	0.5			13.5	0.6	1.3	1.4	43.4	53.2	15.5
	5	320	Flagellates ++	26.1	1.6	1.1	3.2	31.6	62.5	21.1
	10			2.9	1.5	6.5	1.9	12.1	78.1	10.0
June 11	0.5			7.5	1.6	2.7	1.1	28.9	64.9	22.9
	5	289	Thalassiosira rotula	7.0	5.3	1.4	1.5	35.9	55.9	22.9
	10		Skeletonema costatum +	22.5	1.3	0.9	1.2	34.0	62.5	10.1
June 23	0.5		Thalassiosira rotula	11.4	0.6	0.1	0.3	70.0	25.9	16.4
	5	343	Skeletonema costatum	10.1	1.7	1.0	5.0	66.4	24.9	25.8
	10		Flagellates +	4.1	10.0	0.1	1.0	25.0	62.9	10.3
July 16	0.5		Thalassiosira rotula	13.8	1.0	0.2	2.1	63.4	33.3	18.7
	5	476	Skeletonema costatum	1.6	4.2	1.2	1.7	28.6	64.4	13.5
	10		Flagellates	1.1	1.9	0.9	1.5	28.8	66.9	9.3

+ major species

++ 50% of cell numbers

* % of ^{14}C fixed

TABLE 3.2

Distribution of ^{14}C-photosynthate in Indian Arm phytoplankton sampled and incubated for 4 hr from 10:00 a.m. to 2:00 p.m. at 0.5 and 12 m. Half the surface sampler were exposed to normal surface light intensities. Neutral density filters were used to expose the remaining surface samples to light intensities approximately the same as intensities at 12 m.

						Percentage of ^{14}C recovered in the phytoplankton					
						Soluble in					
Date 1969	Sample	Total incident radiation langleys/4hr	Principal cell groups	Primary production mg C/m^3/hr	Salinity ‰	Petroleum ether	0.2 N PCA	Boiling 10% TCA	Ethanol soluble	Insoluble residue	Excreted* organic carbon
	0.5 m^h		Melosira sp +	6.29	16.86	2.8	1.9	1.5	45.1	48.6	18.4
Mar 22	0.5 m^l	86	Skeletonema costatum			0.9	4.3	3.6	61.3	29.8	27.2
	12 m		Flagellates	1.01	27.13	1.1	3.2	7.4	25.3	62.9	12.3
	0.5 m^h		Melosira sp +	2.96	16.27	2.1	1.5	2.5	46.7	45.1	18.3
Apr 10	0.5 m^l	126	Skeletonema costatum			1.1	0.8	1.8	65.3	31.1	31.1
	12 m			1.51	26.81	0.7	2.3	4.8	15.8	76.3	9.6
	0.5 m^h		Skeletonema costatum+	0.55	14.86	2.2	3.1	1.5	40.8	52.3	21.2
Apr 25	0.5 m^l	143	Thalassiosira sp			3.5	0.9	3.7	42.6	49.3	23.8
	12 m			0.06	25.26	0.8	1.1	0.5	32.1	65.3	11.3
	0.5 m^h		Thalassiosira sp +	9.47	13.14	1.0	6.3	1.6	40.8	51.1	21.3
May 8	0.5 m^l	173	Chaetocerus sp			1.2	0.8	0.4	58.1	39.6	26.3
	12 m		Flagellates	1.67	21.83	4.2	2.1	2.2	21.7	69.8	11.2
	0.5 m^h		Thalassiosira sp +	4.59	12.16	4.2	5.1	3.1	61.3	26.3	26.8
May 15	0.5 m^l	186	Chaetocerus sp			0.9	8.1	1.4	68.2	21.4	27.3
	12 m		Flagellates	1.30	24.00	1.8	1.3	3.5	24.3	69.1	10.6
	0.5 m^h		Synedra sp	16.31	12.98	0.7	0.3	0.1	68.3	30.6	21.6
June 2	0.5 m^l	198	Thalassiosira sp			1.8	0.3	0.2	61.9	35.9	34.2
	12 m		Flagellates +	3.56	23.31	1.1	4.6	1.3	21.7	71.3	10.3
	0.5 m^h		Synedra sp	10.03	14.90	1.1	0.7	1.6	61.9	34.7	21.4
June 16	0.5 m^l	184	Thalassiosira sp			1.8	1.1	0.9	67.3	28.9	19.9
	12 m		Flagellates	1.47	21.25	2.1	3.9	1.1	32.2	59.8	9.7
	0.5 m^h			8.69	15.89	1.9	3.7	1.8	67.3	25.3	23.2
June 25	0.5 m^l	205	Flagellates ++			1.0	1.2	5.4	45.1	47.4	19.6
	12 m			2.31	24.13	2.1	2.3	3.7	29.1	62.8	11.2
	0.5 m^h		Synedra sp	5.54	17.10	1.0	0.9	2.1	68.4	27.6	18.4
July 5	0.5 m^l	203	Flagellates +			3.1	2.6	2.3	63.1	28.9	20.7
	12 m			3.46	24.31	0.7	2.0	2.4	33.2	61.7	7.3

\+ major species

++ 50% of cell numbers

* % ^{14}C fixed

h phytoplankton exposed to incident light intensities

l phytoplankton exposed to light intensities reduced to approximately the intensities at 12 m

	0.5 m	5 m	10 m
ethanol-soluble fraction	205.7	162.5	99.9
insoluble fraction	177.3	207.7	270.4

On May 23 and June 11 the pattern of ^{14}C distribution differed from the general pattern. Activity in the insoluble fraction was higher than the soluble fraction. However the ^{14}C in the insoluble fraction was usually higher than in the ethanol-soluble fraction in the 5 m samples and was consistently higher at 10 m (Table 3.1).

Although the insoluble fraction was not routinely analyzed, acid hydrolysis of this fraction on two occasions yielded large quantities of ^{14}C-labelled amino acids. Bidwell (1957) and Bidwell, Craigie and Krotkov (1958) have shown that the insoluble fraction in algae contains primarily proteins and some polysaccharides.

The percent of total activity in the insoluble fraction in Indian Arm surface phytoplankton exposed to either surface or artificially lowered light intensities, was usually low compared with that in the ethanol-soluble fraction (Table 3.2). However, the two fractions contained approximately the same proportion of the total ^{14}C in the March and April samples exposed to the surface but not the lowered intensities. The proportion of ^{14}C in the insoluble fraction was consistently higher in the 12 m samples than in surface samples exposed to

either high or lowered intensities. Conversely, the ^{14}C activity in the ethanol-soluble fraction was lower in 12 m samples than in the two surface samples.

Distribution of ^{14}C within the ethanol-soluble fraction from Saanich Inlet and Indian Arm populations is given in Tables 3.3 - 3.7. At 0.5 m ^{14}C activity in the carbohydrates of the Saanich Inlet populations was usually greater than 50% of the total ethanol-soluble activity (Table 3.3). The relative importance of carbohydrates generally decreased with depth. At 10 m the percentages of ^{14}C in carbohydrates ranged from 13.9 - 21.9%. No consistent trend was evident in the carbohydrate fraction at 5 m. The decrease in relative activity in the carbohydrate fraction with depth is more apparent in Table 3.4 where the percent activity in each compound at each depth is presented as a total for the four sets of experiments. The proportion of ^{14}C in the carbohydrate fraction of surface Indian Arm phytoplankton, whether exposed to surface or artificially lowered intensities, was higher than in the 12 m samples (Table 3.5). In both the Saanich Inlet and Indian Arm phytoplankton the sugar phosphates and glucose contained the greatest proportion of the carbohydrate activity.

The relative proportion of ^{14}C in amino acids increased with depth (Tables 3.3, 3.4, 3.6). At 0.5 m the amino acid activity in Saanich Inlet populations ranged from 18.6 - 43.5%

TABLE 3.3

Distribution of ^{14}C within the ethanol-soluble fraction in Saanich Inlet phytoplankton sampled and incubated at 0.5, 5 and 10 m

		PERCENTAGE OF ^{14}C RECOVERED IN THE VARIOUS FRACTIONS																														
		AMINO ACIDS													ORGANIC ACIDS										CARBOHYDRATES								
DATE	DEPTH (m)	asp	glut	ser	gly	asp-N	glut-N	ala	arg	prol	val	leuc	orn	unknown	total	suc	fum	mal	cit	oxal	acon	gly	& ketoglut	unknown	total	sugar-p	gluc	frug	suc	raf	glycerol	mannitol	unknown	total
May 21	0.5	1.4	1.6	0.6	0.6	2.2	8.0	4.8	3.8	2.9	1.3	0.9	–	0 8	43.5	0.5	0.4	0.4	0.6	–	–	–	–	3.0	4.9	9.4	31.8	–	1.7	2.2	–	–	6.3	51.6
	5.0	2.7	7.5	1.2	1.1	1.2	2.0	27.5	1.3	8.1	1.6	7.9	–	1.5	63.5	–	0.6	0.8	–	0.9	1.3	4.8	–	3.3	11.8	7.9	3.0	4.0	2.2	–	3.9	–	3.6	24.7
	10.0	9.0	21.6	2.1	1.5	3.2	0.3	3.7	1.7	16.6	4.0	4.5	–	3.5	71.8	0.6	0.6	0.4	–	–	1.4	0.8	–	1.3	15.5	3.4	3.1	1.7	2.7	–	2.0	–	1.0	13.9
June 11	0.5	10.5	1.4	1.3	0.6	1.1	1.8	6.2	1.2	4.6	3.9	–	0.9	1.7	35.3	3.6	–	–	2.8	–	–	7.2	–	7,1	20.8	2.2	3.9	–	0.9	–	1.9	1.0	1.7	11.6
	5.0	1.1	6.4	0.4	0.7	–	0.4	0.8	–	1.0	9.5	–	–	1.4	21.8	7.5	2.2	7.7	–	–	–	–	–	19.8	49.1	12.1	6.8	1.7	0.5	–	1.8	–	6.2	29.2
	10.0	1.5	9.1	2.3	0.7	2.7	3.5	3.1	2.9	2.6	8.2	3.7	0.7	1.9	42.9	2.6	3.9	3.4	13.2	–	–	–	–	12.0	35.2	6.8	3.8	4.7	2.2	–	–	–	4.3	21.9
June 23	0.5	3.2	0.5	0.5	0.5	0.7	0.7	0.9	0.9	0.9	4.1	5.0	–	10.7	18.6	–	6.8	1.6	–	–	0.6	0.7	–	5.2	14.4	17.1	33.3	0.7	3.5	3.9	0.9	–	7.6	67.0
	5.0	6.6	3.0	0.5	0.5	0.9	0.6	3.5	0.8	2.1	0.3	0.4	–	1.8	21.1	0.1	0.2	0.2	0.3	1.3	–	0.4	–	0.7	3.2	3.2	60.1	1.7	2.1	3.1	0.6	1.3	3.5	75.7
	10.0	13.0	7.8	2.5	3.1	2.2	3.8	3.6	3.0	2.8	2.6	3.0	–	4.4	51.5	1.4	3.9	3.8	7.2	3.4	–	26.6	–	3.3	26.6	8.3	4.9	3.7	–	–	0.5	1.1	3.2	21.8
July 16	0.5	4.3	3.4	1.0	–	0.7	0.9	1.1	1.3	1.6	1.6	2.5	–	1.8	20.2	4.9	–	–	4.4	1.8	1.2	4.9	1.2	2.9	21.3	28.8	17.5	1.8	–	0.2	3.7	1.1	7.0	58.5
	5.0	11.5	10.6	3.4	2.2	2.9	3.1	3.9	0.9	–	4.9	4.2	–	3.6	51.4	–	2.8	–	3.6	4.1	–	3.0	1.7	4.6	19.9	14.2	7.6	1.8	0.9	1.5	–	0.9	1.8	19.4
	10.0	43.9	6.1	3.3	1.6	1.9	2.3	8.9	1.7	0.9	3.0	1.9	–	2.0	77.3	2.3	0.8	1.5	–	–	–	1.8	–	0.8	7.2	7.3	2.7	1.9	0.8	0.8	–	–	1.9	15.5

TABLE 3.4

The percent ^{14}C activity in various amino acids, organic acids and carbohydrates presented as a total for each component at each depth over the four sets of experiments in Saanich Inlet from March 22 to July 16, 1968.

Amino acids	0.5m	5m	10m
aspartate	19.4	21.9	67.4
glutamate	6.9	27.5	44.6
serine	3.4	5.5	10.2
glycine	1.7	4.5	6.9
asparagine	4.7	5.0	10.0
glutamine	11.4	6.1	9.9
alanine	13.0	35.7	19.3
arginine	7.2	3.0	9.3
proline	10.0	11.2	22.9
valine	10.9	16.3	17.8
leucine	8.4	12.5	13.1
ornithine	0.9	–	0.7
total	117.6	106.4	243.5

Organic acids	0.5m	5m	10m
succinate	9.0	7.6	6.9
fumarate	7.2	5.8	9.2
malate	2.0	8.7	9.1
citrate	7.8	3.9	20.4
oxalate	1.8	6.3	3.4
aconitic	1.8	1.3	1.4
glycolate	12.8	8.2	6.2
α ketoglutarate	1.2	1.7	–
total	61.4	84.0	84.5

Carbohydrates	0.5m	5m	10m
sugar-phosphates	57.5	37.4	25.8
glucose	86.5	77.5	14.5
fructose	2.5	9.2	12.0
sucrose	6.1	5.7	5.7
raffinose	6.3	4.6	0.8
glycerol	6.5	6.3	2.7
mannitol	2.1	2.4	1.1
total	167.5	143.1	62.6

TABLE 3.5

Distribution of ^{14}C among carbohydrates in the ethanol-soluble fraction of Indian Arm phytoplankton after 4 hr. incubation

Date 1969	Sample	Percentage of ^{14}C recovered in the ethanol-soluble fraction											
		sugar-P	gluc	fruc	gal	raff	man	eryth	suc	glycerol	mannitol	unknowns	totals
Mar 22	0.5 m^h												40.3
	0.5 m^l	lost											61.6
	12 m												28.5
Apr 10	0.5 m^h	24.2	16.3	1.3	–	1.5	1.9	1.0	2.7	–	3.9	9.3	62.1
	0.5 m^l	34.9	28.6	6.1	1.5	–	1.0	–	–	–	–	8.0	80.1
	12 m	18.7	9.5	–	–	1.3	2.1	–	3.0	–	2.3	5.7	42.6
Apr 25	0.5 m^h	13.3	19.3	1.1	–	–	4.8	–	4.1	–	–	3.3	45.9
	0.5 m^l	14.1	5.8	2.6	–	1.7	1.7	2.8	4.9	0.8	2.2	17.4	53.9
	12 m	10.3	8.6	3.1	1.8	–	2.3	–	3.3	–	–	2.7	32.1
May 8	0.5 m^h	26.2	19.1	3.4	1.9	1.0	–	–	2.3	4.7	–	8.2	66.9
	0.5 m^l	7.4	20.4	6.1	–	12.2	–	1.3	3.8	–	2.0	10.1	56.1
	12 m	12.9	3.8	2.0	–	2.1	–	–	2.9	–	0.5	2.3	26.5
May 15	0.5 m^h	11.6	8.1	2.6	6.0	2.4	1.0	1.6	8.1	–	3.0	2.5	46.9
	0.5 m^l	14.9	9.5	2.3	6.9	3.1	1.9	–	5.3	1.1	2.4	3.2	50.6
	12 m	3.0	5.1	–	2.8	2.3	–	2.1	4.3	–	2.1	1.1	22.8
June 2	0.5 m^h	23.1	18.6	2.2	0.9	–	4.5	6.3	7.4	1.9	–	4.1	69.0
	0.5 m^l	29.9	13.8	1.6	1.1	–	3.9	5.2	3.3	1.3	0.9	5.4	66.4
	12 m	6.1	11.8	1.1	–	0.8	1.0	–	1.4	–	1.1	1.4	24.7
June 16	0.5 m^h	23.6	9.5	3.3	1.6	0.8	2.8	4.4	6.1	–	0.5	3.8	56.4
	0.5 m^l	18.7	8.3	2.9	2.6	1.1	4.2	1.3	3.1	0.9	1.8	4.9	49.8
	12 m	6.3	3.1	4.2	0.9	–	1.3	–	–	–	–	2.9	19.3
June 25	0.5 m^h	17.1	12.2	1.9	3.8	1.7	2.7	3.0	0.9	3.2	–	3.1	49.8
	0.5 m^l	22.3	12.2	3.7	5.1	0.9	2.3	3.5	4.1	–	2.9	–	57.3
	12 m	9.3	2.2	2.9	1.1	0.9	1.5	1.5	3.3	–	–	2.1	26.2
July 5	0.5 m^h	18.1	11.0	2.1	0.9	1.1	–	–	1.4	–	–	3.9	38.5
	0.5 m^l	29.7	6.2	8.6	–	1.1	2.3	1.7	3.9	–	2.1	2.1	57.8
	12 m	6.2	3.3	1.1	1.1	2.1	0.9	1.7	2.8	1.1	2.7	2.7	22.7

h phytoplankton exposed to incident light intensities

l phytoplankton exposed to light intensities reduced to approximately the intensities at 12 m

TABLE 3.6

Distribution of ^{14}C among amino acids in the ethanol-soluble fraction of Indian Arm phytoplankton after 4 hr incubation

Date 1969	Sample	Percentage ^{14}C recovered in the ethanol soluble fraction														
		asp	glut	gly	ser	asp-N	glut-N	ala	arg	prol	val	leuc	meth	orn	unknown	total
Mar 22	0.5 m[h]	9.3	6.3	4.2	3.9	1.9	0.9	5.1	1.1	8.6	2.7	–	–	2.1	1.4	47.1
	0.5 m[l]	7.3	4.2	1.1	0.8	1.0	0.8	7.2	1.3	2.7	–	1.1	–	1.7	1.9	30.1
	12 m	6.2	3.9	5.3	2.7	2.9	0.8	3.1	4.8	12.0	1.6	2.3	1.2	1.4	2.1	50.3
Apr 10	0.5 m[h]	2.1	3.3	0.9	1.2	–	1.1	8.3	1.0	–	3.2	1.3	–	1.3	1.1	24.8
	0.5 m[l]	1.1	2.2	0.6	–	0.9	–	0.4	1.4	3.7	1.1	–	–	–	0.9	12.3
	12 m	7.3	6.0	7.9	0.9	–	–	17.9	–	–	–	–	0.6	–	1.5	41.9
Apr 25	0.5 m[h]	12.2	7.2	0.6	0.6	–	–	1.0	6.3	1.5	0.7	0.7	–	2.1	1.9	34.8
	0.5 m[l]	3.6	1.4	–	–	0.9	–	7.4	1.0	1.3	–	–	–	2.4	2.6	20.6
	12 m	18.4	6.9	2.4	1.3	–	3.7	0.5	–	4.8	1.6	0.5	–	–	0.9	40.8
May 8	0.5 m[h]	3.1	–	0.5	–	–	–	7.3	1.1	–	–	3.8	–	–	2.8	17.6
	0.5 m[l]	1.0	5.8	1.2	2.2	–	–	1.8	1.0	4.1	2.4	–	–	–	1.7	21.2
	12 m	14.4	10.7	–	1.1	0.7	4.1	–	1.2	16.6	–	7.8	–	1.1	4.6	62.3
May 15	0.5 m[h]	3.9	4.6	2.6	–	2.1	1.8	2.9	–	1.9	–	2.7	–	–	2.4	24.9
	0.5 m[l]	4.0	3.8	2.5	–	1.7	1.5	2.0	0.9	–	1.1	2.0	–	–	3.6	23.1
	12 m	5.0	10.9	2.0	1.5	–	4.5	5.4	2.0	1.8	–	2.8	0.9	–	1.0	34.8
June 2	0.5 m[h]	2.6	3.9	–	–	0.8	–	2.7	–	1.5	1.0	–	–	1.2	1.1	14.8
	0.5 m[l]	3.1	3.3	1.0	–	0.9	–	1.9	–	1.7	1.4	–	–	1.4	2.3	17.0
	12 m	13.1	2.3	3.1	2.1	1.0	0.9	9.6	3.1	12.2	3.7	2.6	1.1	1.7	1.9	58.3
June 16	0.5 m[h]	4.2	6.0	1.6	1.6	1.5	–	1.2	1.0	1.2	–	0.8	0.7	–	1.4	21.2
	0.5 m[l]	4.0	9.2	0.9	1.1	0.9	–	2.3	1.7	1.0	1.1	1.9	–	–	0.9	25.7
	12 m	12.9	8.1	7.2	4.3	3.1	4.2	9.1	3.3	6.3	3.1	–	–	–	2.6	62.2
June 25	0.5 m[h]	6.1	4.2	1.0	2.3	1.1	–	1.1	–	1.4	2.0	1.8	–	1.7	1.1	23.8
	0.5 m[l]	4.3	3.9	0.9	1.4	–	1.8	1.3	–	1.9	1.7	1.9	–	–	0.8	19.1
	12 m	16.6	12.1	3.3	4.2	0.9	1.1	5.0	3.4	3.1	8.3	1.0	–	–	2.3	61.3
July 5	0.5 m[h]	7.9	4.2	1.3	2.2	1.9	0.8	6.1	–	9.2	2.1	–	1.7	–	2.7	40.1
	0.5 m[l]	4.1	6.2	1.3	2.1	–	0.8	2.3	–	3.1	2.3	–	0.9	1.6	2.2	26.9
	12 m	16.8	12.1	3.1	4.7	8.6	1.4	3.4	2.9	3.7	2.9	1.9	1.2	–	1.5	64.2

h phytoplankton exposed to incident light intensities

l phytoplankton exposed to light intensities reduced to approximately the intensities at 12 m

of the total in the ethanol-soluble fraction. With one exception, the relative activity at 5 m was higher than at the surface and was consistently higher at 10 m than in the upper two sampling depths. The amino acids, aspartate and glutamate usually contained the greatest proportion of ^{14}C. Both increased in relative importance with depth (Table 3.3). Alanine, proline, serine, valine and asparagine, which occasionally contained significant proportions of the relative activity, showed the same trend. Similarly, the proportion of ^{14}C in the amino acid fraction in Indian Arm phytoplankton was higher in the 12 m samples than in surface samples exposed to either in situ or artifically lowered intensities. The depth-dependent increase was apparent in aspartate and glutamate, and usually in serine, alanine, proline and valine.

No clear changes are apparent in the distribution of ^{14}C among the organic acids in either Saanich Inlet or Indian Arm phytoplankton (Tables 3.3, 3.4, 3.7). However, relative ^{14}C activity in the organic acid fraction was generally higher in the samples from the lower part of the photic zone than in the surface samples exposed either to the in situ or artificially lowered intensities.

The percent of ^{14}C excreted by the phytoplankton relative to the ^{14}C recovered in the cells was consistently lower in phytoplankton from the lower part of the photic zone (Tables 3.1,

TABLE 3.7

Distribution of ^{14}C among organic acids of the ethanol-soluble fraction of phytoplankton after 4 hr incubation

Date 1969	Sample	Percentage of ^{14}C recovered in the ethanol-soluble fraction										
		suc	fum	mal	cit	oxal	acon	glyc	∝ keto glut	glyceric	unknown	total
	0.5 m[h]	lost										12.6
Mar 22	0.5 m[l]	–	2.4	1.7	0.9	1.0	–	1.2	–	1.1	1.2	8.3
	12 m	1.1	1.9	6.2	1.5	1.5	1.0	4.2	–	2.1	1.7	21.2
	0.5 m[h]	2.9	0.9	–	3.2	0.8	–	1.9	1.5	–	1.9	13.1
Apr 10	0.5 m[l]	1.4	–	2.1	1.0	–	–	1.9	–	–	1.1	7.5
	12 m	3.7	3.5	–	1.0	–	1.1	2.8	–	–	3.5	15.5
	0.5 m[h]	3.2	–	5.8	2.7	3.1	1.1	2.0	–	–	1.3	19.2
Apr 25	0.5 m[l]	–	2.4	0.9	1.0	2.3	–	12.0	3.4	–	3.0	25.5
	12 m	lost										27.0
	0.5 m[h]	1.1	2.0	2.1	1.4	–	–	1.9	–	2.0	3.1	15.5
May 8	0.5 m[l]	5.3	2.0	6.3	1.7	2.2	–	2.8	–	2.5	5.3	28.2
	12 m	2.1	4.1	–	–	–	–	3.1	–	–	2.0	11.3
	0.5 m[h]	1.7	1.3	1.6	3.3	2.1	5.8	6.3	2.8	–	3.4	28.2
May 15	0.5 m[l]	2.1	1.0	0.9	2.5	2.3	4.2	4.4	2.3	2.5	4.1	26.3
	12 m	7.4	1.5	9.5	3.5	2.1	3.6	3.2	3.5	1.8	6.3	42.4
	0.5 m[h]	2.4	1.1	3.8	0.9	1.0	1.7	1.4	–	1.8	2.0	16.1
June 2	0.5 m[l]	2.0	1.5	2.6	1.4	–	1.0	0.8	2.0	2.1	3.1	16.5
	12 m	1.1	0.9	3.1	1.9	2.4	2.5	1.3	2.0	–	1.9	17.1
	0.5 m[h]	4.2	3.6	2.7	1.0	–	1.4	3.1	1.0	0.9	3.3	21.2
June 16	0.5 m[l]	4.9	2.9	3.2	1.8	1.3	1.1	5.5	–	1.1	2.7	25.7
	12 m	2.2	0.9	3.4	2.3	–	2.1	3.9	–	1.6	2.1	18.5
	0.5 m[h]	5.3	3.9	2.3	1.2	2.1	1.1	4.6	–	0.9	2.9	26.4
June 25	0.5 m[l]	3.9	2.7	1.8	–	2.3	2.2	4.0	1.9	1.3	3.5	23.6
	12 m	2.1	2.8	1.5	1.1	–	0.9	1.3	–	0.9	1.8	12.5
	0.5 m[h]	3.2	5.1	2.2	2.1	1.6	0.9	1.3	–	1.9	3.1	21.4
July 5	0.5 m[l]	1.2	3.4	2.3	2.6	0.8	–	2.1	–	–	2.9	15.3
	12 m	2.1	2.7	1.7	3.1	1.2	–	0.8	–	–	1.5	13.1

h phytoplankton exposed to incident light intensities

l phytoplankton exposed to light intensities reduced to approximately the intensities at 12 m

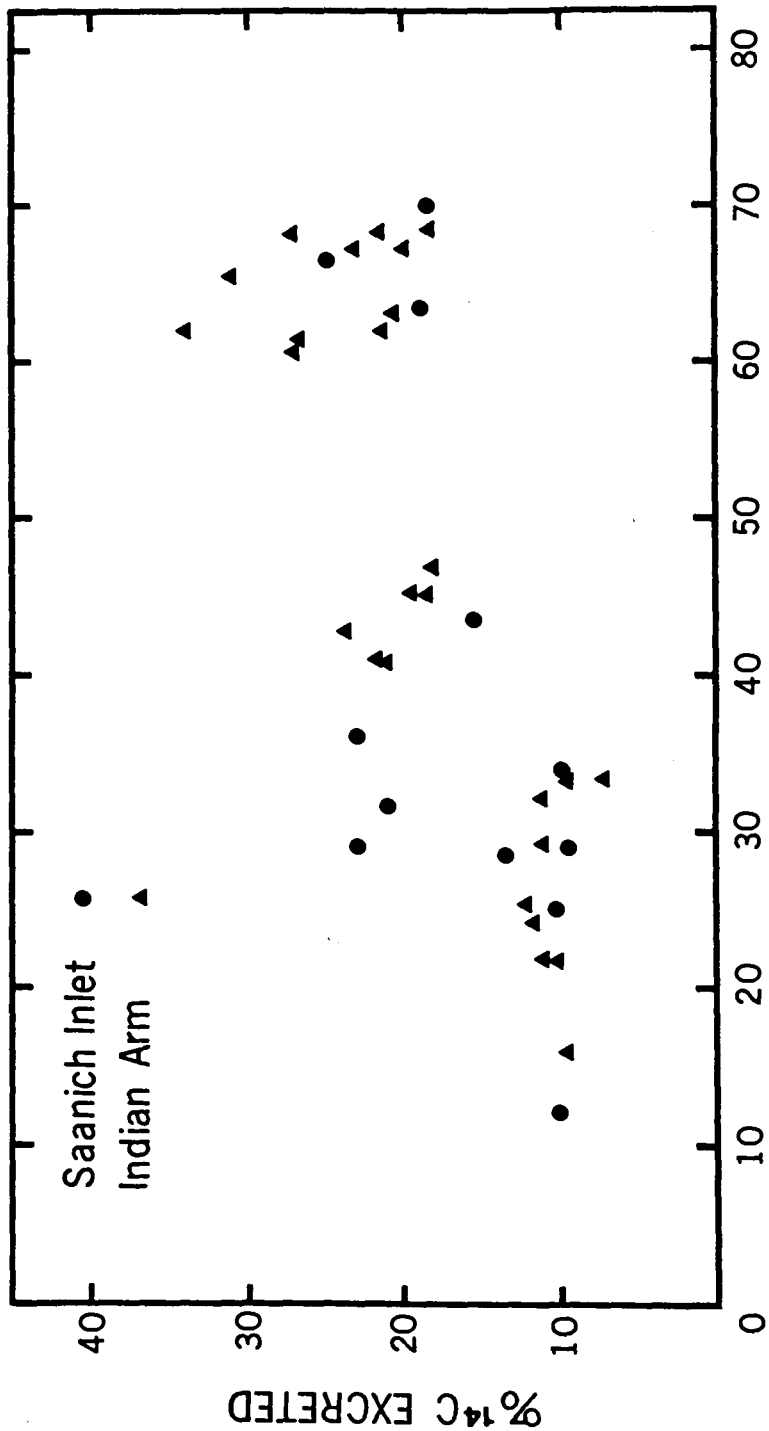
3.2). Loss of labelled organic compounds by Saanich Inlet phytoplankton varied from 13.5 - 25.8% of the ^{14}C fixed at 0.5 and 5 m. Surface phytoplankton from Indian Arm exposed to either *in situ* or artificially lowered intensities lost 18.4 - 34.2% of total fixed activity. Excretion in the lower part of the photic zone was approximately 10%. The proportion of ^{14}C excreted by the phytoplankton tended to increase with an increase in the relative activity in the ethanol-soluble fraction (Tables 3.1, 3.2; Fig. 3.2).

DISCUSSION

The data in Tables 3.1 and 3.2 indicate a depth-dependent shift in the distribution of ^{14}C between the ethanol-soluble and insoluble fractions in Saanich Inlet and Indian Arm phytoplankton. The observed changes in distribution of ^{14}C between the two fractions were associated with a decrease in light intensity and change in quality. The spectral distribution of radiation in the two areas shifted from white light at the surface to predominately blue-green light in the lower part of the photic zones.

These differences in distribution of ^{14}C between the two fractions are in general agreement with the laboratory results on the nature of the photosynthate in plankton algae exposed to

Figure 3.2 The relationship between the proportion of released organic carbon and the proportion of ^{14}C in the ethanol-soluble fraction of phytoplankton from various depths in Saanich Inlet and Indian Arm.


Saanich Inlet
Indian Arm
0
10
20
30
40
10
20
30
40
50
60
70
80
% 14C EXCRETED
% 14C IN THE ETHANOL—SOLUBLE FRACTION

light of different spectral quality but equivalent intensities (Chapter 1). In cultures grown in white light (as at the surface) the greatest proportion of ^{14}C was incorporated into the ethanol-soluble fraction whereas in cultures grown in blue or green light (comparable to the lower part of the photic zone) most of the ^{14}C occurred in the insoluble compounds, primarily proteins. Comparable changes occurred at high and low intensities.

Since the changes in Saanich Inlet phytoplankton populations were similar to those observed in cultures it appears that the depth-dependent shift in the chemical nature of the photosynthate was a response to the spectral quality of radiation although the effects of changes in intensity were not entirely eliminated. The depth-dependent changes in pigment concentrations and photosynthetic rates in phytoplankton reported by other investigators are usually attributed to changes in light intensity (Steemann Nielsen and Jorgensen 1968, Steemann Nielsen and Hansen 1961, Ryther and Menzel 1959) although no particular attention has been given to light quality.

To estimate the effect of differences in light intensities on the distribution of carbon, a second investigation was conducted in Indian Arm in 1969. Phytoplankton from the surface and 12 m were exposed to <u>in situ</u> intensities. In addition, surface phytoplankton were exposed to intensities comparable to

those at 12 m by use of neutral density filters.

The distribution of ^{14}C between the ethanol-soluble and insoluble fractions in Indian Arm surface phytoplankton exposed to *in situ* intensities or artificially lowered intensities was similar to that in surface phytoplankton from Saanich Inlet and in algal cultures grown in white light. The metabolic response of phytoplankton in the lower part of the photic zone in Indian Arm was similar to that in Saanich Inlet and cultures grown in blue or green light. These results are evidence that the depth-dependent changes in the ethanol-soluble and insoluble compounds reflect the responses of phytoplankton to differences in the wavelength of the light.

In field studies extending from March through August, Olive and Morrison (1967) and Olive *et al.* (1969) have demonstrated an increase in the insoluble compounds with depth in freshwater phytoplankton. They suggested that these differences were due to successional changes in the standing crop and to variations in environmental conditions although they did not consider light quality. Their results and those reported here are quite similar which strongly suggest that the main factor producing variations in ^{14}C distribution within the cells was the spectral quality of light.

The variations in ^{14}C among compounds of the ethanol-soluble fraction also indicate a spectral quality response under natural

conditions. For example, the relative activity in some amino acids, in particular aspartate and glutamate increased with depth. This occurred in both Saanich Inlet (Tables 3.3, 3.4) and Indian Arm (Table 3.6). The relative concentration of carbohydrates, principally glucose and sugar phosphates, was higher at the surface than in deep water phytoplankton (Tables 3.3, 3.4, 3.6). These depth-dependent changes, associated with shifts from white to blue-green light, were comparable to those observed in culture studies (Chapter 1) in that the proportion of ^{14}C in amino acids was lower and in carbohydrates was higher in white than in blue or green light.

In the culture studies some organic acids were higher under blue and green light. The total activity in the organic acid fraction was usually higher in deep than in surface phytoplankton populations (Tables 3.3, 3.4, 3.7) although there was no consistent trend in individual organic acids. The organic acids in laboratory cultures were usually higher in blue and green than in white light of the same intensity.

Olive and Morrison (1967) and Olive et al. (1969) have attributed some of these depth-dependent changes in ^{14}C distribution to variations in the rates of photosynthesis. In vascular plants carbohydrates usually increase and proteins decrease with increasing rates of photosynthesis (Krotkov 1960). However, the work on surface algae from Indian Arm showed similar patterns in

^{14}C distribution in cells exposed to high or artificially lowered intensities (Tables 3.2, 3.5 - 3.7). Further, rates of photosynthesis in the algal cultures did not affect the nature of the photosynthate (Chapter 1). This suggests that the differences in photosynthetic rates or light intensities have little effect of the nature of photosynthetic products in natural phytoplankton communities. The shift in ^{14}C distribution with depth is a response to the quality of radiation to which the cells are exposed.

It is known that salinity affects primary production in natural waters (Steemann Nielsen 1964, Nakanishi and Monsi 1965) and in algal cultures (Vosjan and Siezen 1968, Craigie 1969). There was little vertical change in salinity in Saanich Inlet during the course of the study and considerable variation in Indian Arm. The similarity of the data obtained in these field studies suggests that salinity had no obvious effect on the nature of the photosynthate.

These studies with naturally occurring phytoplankton populations indicate that there is a depth-dependent change in the percentage of extracellular organic carbon released by the cells. The percentage released near the bottom of the photic zone was less than half that at the surface. This was observed at both Saanich Inlet and Indian Arm where surface phytoplankton were exposed to <u>in situ</u> as well as artificially lowered light

intensities. There was no significant differences in the proportion of organic carbon excreted by the phytoplankton under high or low intensities of light of the same wavelength. This suggests that alterations in the proportion of organic carbon released at the different depths are associated with differences in light quality.

These depth-dependent differences in release may be a result of the shifts in metabolism associated with differences in light quality. The percentage release of extracellular products was proportional to the percent ^{14}C the phytoplankton incorporated into the ethanol-soluble fraction (Fig. 3.2). The majority of the compounds in the ethanol-soluble fraction are low molecular weight. The quantity of organic carbon released may depend on an equilibrium between the intra- and extracellular concentrations of these compounds (Nalewajko et al. 1963). Pritchard et al. (1963) suggested diffusion governs the release of glycolate from Chlorella vulgaris.

Changes in the percentage of extracellular release in association with light of different wavelength have not previously been reported from field studies. Further, there are no studies correlating release with the nature of the photosynthate. However, Becker et al. (1969) showed an inhibition of glycolate release from Chlorella vulgaris and C. pyrenoidosa in blue light. The results in Chapter 1 show a decrease in

production of ethanol-soluble compounds (of which glycolate is representative) in blue light. This would suggest a decrease in excretion as a function of the percentage of low molecular weight compounds in the photosynthate.

The field investigations have shown that the nature of photosynthetic products changes with depth in natural populations. This suggests that the observed differences can be interpreted as a response by the algae to the wavelength of light to which they are exposed.

In the culture studies differences in concentrations of photosynthetic pigments, growth rates and I_k values were observed under different light quality. Although these features were not examined during the field studies, depth-dependent changes (i.e. light quality) comparable to those outlined in the previous chapters would be expected since alterations in the photosynthetic products were similar to those observed in culture studies. The numerous investigators (Ryther and Menzel 1959, Steemann Nielsen and Hansen 1961, as examples) who observed changes in pigment concentrations and I_k values with depth have usually attributed the changes to differences in light intensity.

The field studies suggest that the shift in metabolism may be fairly rapid when the cells are exposed to light of a different wavelength. The water column was weakly stratified

in Saanich Inlet and strongly stratified in Indian Arm. Considerable mixing occurred in Saanich Inlet. There was, however, a similarity in the nature of the recently formed metabolites at comparable depths in the two localities in spite of a high probability that phytoplankton cells in Saanich Inlet would not exist under stratified conditions for any extended period of time. This suggests that a relatively short time span, perhaps of the order of a few days or less, is required for the effect of changed light quality to be expressed in the nature of the photosynthate.

Differences in the chemical composition of algae which can be expected to vary vertically may influence the composition of breakdown products in different parts of the water column. The observed decrease in percentage release of extracellular compounds may also contribute to differences in dissolved organic compounds with depth. This could produce vertical differences in the capacity of the photic zone to support the heterotrophic growth of various bacteria, algae or marine invertebrates. The chemical nature of the extracellular compounds released by algal breakdown products may also influence the formation of organic aggregates. Riley (1963) has shown that dissolved organic compounds may be converted to particulate form under natural conditions. This particulate material can serve as a food source for zooplankton (Baylor and Sutcliffe 1963).

The shifts in algal metabolism associated with differences in light quality may affect the nutritional value of phytoplankton as a food source. For example, blue light such as occurs at the bottom of the photic zone, may enhance protein synthesis. There is little information on which to base interpretation of the nutritional significance of an increase in protein. However, Monochrysis lutherii, which has a high protein content, was the only organism of a number tested that would support indefinite growth of the crustacean Tigriopus japonicus (Shiraishi and Provasoli 1959) and was the best food source tested for oyster larvae (Davis and Guillard 1958).

It is probable that the shifts in relative concentrations of the photosynthetic pigments that occur in response to changes in light quality or quantity have a selective advantage. The observed changes are usually of a type that will increase the efficiency of utilization of light energy at a given depth and thereby enhance the probability of survival of the population. The changes in the nature of the photosynthate that are associated with shifts in the relative importance of the various photosynthetic pigments may simply be a consequence of pigment composition and have no direct selective advantage. However, it may be there are advantages, as yet unidentified in the observed variations in chemical composition of natural phytoplankton populations.

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APPENDIX A

Algal Medium

Taken from Jones *et al.* (1963) and modified to satisfy diatom requirements.

NaCl	270 g/l
$MgSO_4.7H_2O$	6.6 g/l
$MgCl_2.6H_2O$	5.6 g/l
$CaCl_2.2H_2O$	1.5 g/l
KNO_3	1.0 g/l
KH_2PO_4	0.07 g/l
$NaHCO_3$	0.04 g/l
$NaSiO_3.9H_2O$ *	0.15 g/l
1 M Tris-HCl buffer, pH 7.6	20 ml/l

1 ml/l of trace metal solution containing the following dissolved in 100 ml distilled water

$ZnCl_2$	4.0 mg	$CuCl_2.2H_2O$	4.0 mg
H_3BO_3	60.0 mg	$MnCl_2.4H_2O$	40.0 mg
$CoCl_2.6H_2O$	1.5 mg	$(NH_4)_6Mo_7O_{24}.4H_2O$	37.0 mg

1 ml/l of chelated iron solution 240 mg $FeCl_3.4H_2O$ per 100 ml 0.05 M Na_2EDTA, pH 6.

1 ml/l of vitamin solution containing

thiamine.HCl	500 µg/ml
vitamin B_{12}	2 µg/ml

biotin	1 μg/ml

* The same volume of 0.01 N HCl as that of silicate was added to each liter of medium. The two solutions were added simultaneously to avoid precipitation.

APPENDIX B

Contamination Checks

The technique of selective enrichment of possible microbial contaminants (bacteria and molds) by heterotrophic growth in a seawater medium enriched with organic material was used for checking algal cultures for such contamination (Antia and Kalmakoff 1965). The STP medium of Provasoli et al. (1957) was used for such sterility checks. From 2 - 4 drops of algal culture were added to a tube and incubated about 3 weeks at room temperature (20 - 25 C) for a sterility check. The appearance of cloudiness and turbidity typical of bacterial growth or discolouration and tuft-formation typical of mold growth was taken as evidence of possible contamination subject to confirmation by repeat checks. In the absence of any visible changes in the sterility check tube, the algal culture tested was considered to be free from contamination. Periodic sterility checks were routinely maintained on all algal stock cultures. Every stock culture used to provide inoculum for an experimental culture was subjected to such a check in duplicate tubes. Each experimental culture was submitted to such a check (in quadruplicate tubes) just before use in an experiment. All such checks have indicated absence of contamination.

Medium for bacterial contamination check.

To 800 ml HA-Millipore filtered sea water, in a 1500 ml Erlenmeyer flask, add the following in the order indicated:

distilled H_2O	150 ml	
$NaNO_3$	200 mg	
K_2HPO_4	10 mg	
L-glutamate	435 mg	(= 50 mg Na-H-glutamate)
glycine	100 mg	
DL-alanine	100 mg	
Trypticase	200 mg	
Yeast autolysate	200 mg	
Sucrose	1 g	

Shake well to obtain a clear a solution as possible.

add soil extract	50 ml	
vitamin mix	1 ml	

Stir (magnetic) to obtain clear solution. If clear solution is not obtained, filter (Millipore).

Measure pH of clear filtrate, adjust pH with 1 N NaOH to 7.4 - 7.6.

Place 2.5 ml aliquots into 16 x 100 mm screw cap culture vials.

Autoclave 20 min at 100 C and 15 psi.

Cool and store culture flasks and stock solution in store room.

Composition of vitamin mix solution.

Add the following to 100 ml distilled H_2O in 150 ml Erlenmeyer flask.

thymidine	20 mg
nicotinic acid	10 mg
putrescine.2HCl	4 mg
Ca pantothenate	10 mg
riboflavin	500 µg
pyridoxine.2HCl	4 mg
pyridoxamine.2HCl	2 mg
p-aminobenzoic acid	1 mg
biotin	50 µg
choline H_2 citrate	50 mg
inositol	100 mg
thymidine	80 mg
orotic acid	26 mg
B_{12}	5 µg
folic acid	250 µg
folinic acid	20 µg

In order to avoid weighing and to overcome problem of weighing quantities less than 2 mg, 1) prepare a concentrate of p-aminobenzoic acid, riboflabin, folic acid and folinic acid, 2) make up stock concentrates of B_{12}, thiomine, biotin for use.

1) Concentrate of riboflavin, p-aminobenzoic acid, folic acid, folinic acid.

The following amounts of the vitamins were dissolved in 500 ml distilled H_2O in a 1-l Erlenmeyer flask by prolonged warming on a steam bath.

riboflavin	50 mg
p-aminobenzoic acid	100 mg
folic acid	25 mg
folinic acid	2 mg

Note: A clear solution was not obtained on cooling, however, the fine suspension was used for taking 5 ml aliquots for the vitamin mix.

2) Thiamine concentrate: concentration 10 mg/ml

Dissolve 500 mg thiamine.HCl in 50 ml distilled H_2O.

Store in polyethylene bottle at -20 to -30 C, allow to warm to room temperature before taking aliquots.

Use 5 ml aliquots for every 100 ml of total vitamin mix.

3) Biotine concentrate

Weigh out 2.0 mg biotin in 100 ml volumetric flask and make up to volume with distilled H_2O.

Shake and dissolve to give a clear solution by heating flask in hot water (60 - 80 C).

Cool and store in polyethylene bottle at -20 to -30 C, allow to warm to room temperature before taking aliquots.

Use 50 ml aliquots for every 100 ml of total vitamin mix.

4) Vitamin B_{12} concentrate

Dissolve 20 mg crystalline vitamin B_{12} in 50 ml distilled H_2O store in polyethylene bottles at -20 to -30 C, allow to warm to room temperature before taking aliquots.

Use 5 ml aliquots for every 100 ml of total vitamin mix.

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