The Effect of Light of Different Intensities, Reduced Selectively and Non-selectively, upon the Rate of Growth of *Nitzschia closterium.*

By

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With 7 Figures in the Text.

Introduction.

The effect of light upon plant growth is a problem which has attracted many workers since the discovery of Priestly, in 1771, that a plant could purify fixed air, carbon dioxide, which was followed by the supplementary work of Ingen-Housz and Senebier, who showed that the phenomenon was associated with the nourishment of the plant, and took place only in sunlight and through the agency of the green portions of the plant.

Senebier, 1782, was perhaps the first to attempt to show the effect of the different parts of the spectrum on the process, and found that red light was almost as active as ordinary light in the process of photosynthesis whilst the blue was almost inactive. Draper and Daubeny, 1844, concluded that after white light, the red-yellow portion possessed the highest efficiency, and this was supported by Sachs in 1868. Englemann, Timiriazeff and Wolkaff, however, found that whilst the activity in the red was high, there was an important secondary maximum of efficiency in the blue, and these results have been subject to great controversy amongst later workers.

It must be borne in mind that the physics of the light used by the early investigators was not thoroughly understood, and one of the greatest criticisms brought against these workers is that the blue light used by them contained parasitic rays of long wave lengths, viz. in the red region. Dangeard (1927) asserts that in light of feeble intensity, these parasitic rays are practically inactive, but in light of greater intensity they play a highly important role, and are the cause of the secondary maximum observed by many workers. Richter (1902) using coloured screens, concluded that the rate of photosynthesis was proportional to the amount of energy absorbed, independent of the part of the spectrum and the wave length of the ray, and Kniep and Minder (1909) determined that the action of the red and the blue was almost similar when the energy in those regions was the same.

Klugh (1925) sought to determine whether photosynthesis was a wave length phenomenon, or dependent upon the total light intensity. To do this he grew unicellular algæ, in light which he regarded to be of the same intensity but of different wave lengths, and took the rate of reproduction as criteria for efficiency of the wave lengths concerned in photosynthesis, for although the method was indirect it was assumed that, fundamentally, the rate of reproduction in chlorophyllous organisms is dependent on the ability to manufacture food. From a single series of experiments lasting from August 16th–September 10th he concluded that photosynthesis is a wave length phenomenon, red light being highly efficient, blue much less so, and green inefficient, but Klugh himself in his concluding remarks says "that it is dangerous to draw conclusions from a single series of experiments."

Using somewhat similar methods to those of Klugh, results are now presented in this paper, which would show that photosynthesis, as indicated by the rate of growth of marine diatoms, is a function of the amount of energy transmitted independent of the wave length between which the energy so transmitted lies.

Plant material used.

Persistent cultures of the marine diatom *Nitzschia closterium*, grown in "Miquel sea water," were used for the plant growths to be studied, a persistent culture, as defined by Allen and Nelson (1910), being one in which only one species of diatom was present, although there might be bacteria.

It is of interest to mention here, that marine diatoms exhibit well-marked periodicity in the sea, the diatom outburst being at its height between early March and late April. The cause of these outbursts is not fully understood.

Marshall and Orr (1928) concluded that the length of day was a factor of the greatest importance, whilst Atkins, Herdman, Scott and Dakin correlated the early spring sunshine with the diatom outburst. Atkins (1928) also found that "a study of the phosphate change affords a measure, in an inverse ratio, of the production of the algal crop, and indicates from year to year the variations that occur in its seasonal waxing and waning."

From results obtained using the marine diatom *Nitzschia closterium* it would appear that the total light intensity is a factor which has a profound effect upon growth.

METHOD.

It was desired to determine the effect of light of selected wave lengths and of daylight reduced in intensity non-selectively, upon the rate of growth of *Nitzschia closterium*, and this was done by growing cultures of Nitzschia under a series of selective and non-selective filters.

Equal volumes of "Miquel sea water," contained in sterilized crucibles of white porcelain of 10 cubic centimetres capacity and 3·5 centimetres

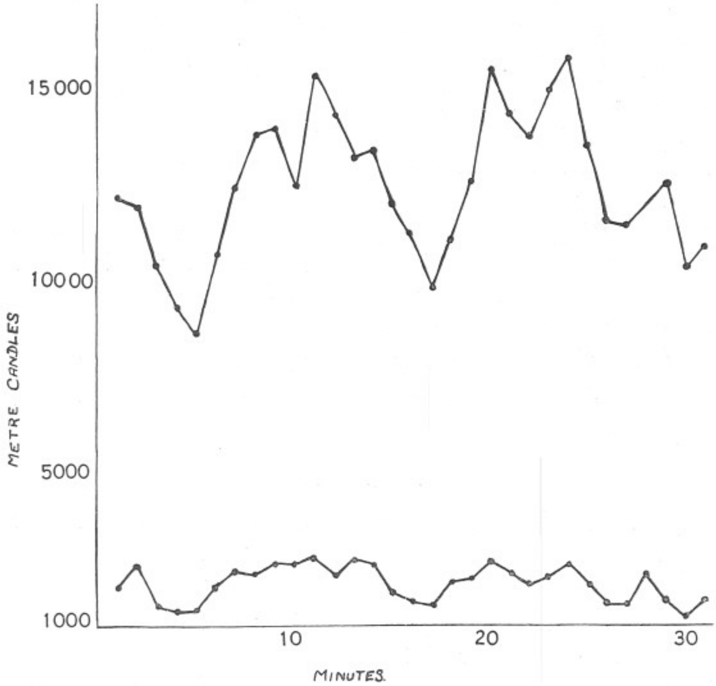

FIG. 1.—Curves showing the variations in illumination for the north window, lower curve, where the cultures were set, and for an open site on the laboratory wall, upper curve, determined simultaneously for a period of 30 minutes. The numbers along the ordinates represent the illumination in metre candles. Those along the abscissæ the time in minutes.

diameter, were inoculated with similar drops of a persistent culture solution, which contained known numbers of diatoms per unit of volume.

The number of diatoms in any culture was determined with the aid of a hœmacytometer, the millimetre square being ruled in Thoma pattern. The culture to be examined was first thoroughly mixed by means of a fine sterilized pipette, this process also serving to aërate the culture. A drop of the mixture was then placed upon the hœmacytometer, and the number of diatom cells per unit of volume, 0·1 cubic millimetre, was then found by counting. For each reading an average of four counts was made, a difference of ten diatoms being allowed for any accepted count.

Examination of the tables of results as a whole, will show that it would have been better to have used larger initial number of diatom cells since their minute size renders them very difficult to count when present in small numbers. This explains the irregularities in the early counts for the cultures of each series.

The sub-cultures were covered with the selected filters and then placed side by side in the window of a north room. The distance between the two end cultures was 80 centimetres. Opposite the window and 4·2 metres away from it, is a high grey wall of limestone, which receives direct sunshine only for a short time as the sun sets.

On 27.2.30, simultaneous measurements were made of the illumination of the window in the north room (V_s) and of a perfectly open site on the laboratory roof (V_o) by means of photometers furnished with vacuum photo-electric cells. (For method see Atkins and Poole, 1929.) It has been shown that on a dull day, the illumination at a point inside a building bears a very nearly constant ratio to the illumination at a point outside, and this ratio, expressed as a percentage, has been termed the daylight factor δ. Table I shows these values over a period of half an hour.

TABLE I.

February 27, 1930. Grey sky, completely overcast, wind N.E. 12.14–12.43 p.m. G.M.T.

Indoor site is the window-sill of a north room, where the photometer is moved over a range of 80 cm. The outdoor site is a fixed position on a parapet of the Laboratory roof.

Indoor sites (in window sill).		G.M.T.	Illumination in meter candles. In open on Roof Vo.	Illumination in meter candles. In window sill Vs.	Daylight Factor. $\frac{Vs.}{Vo.} = \delta$	Average δ
Site 1.	1.	12.14	12,000	1,950	16·2	
Right extremity	2.	12·16	10,200	1,490	14·6	
occupied by	3.	12·17	9,150	1,389	15·2	
cultures.	4.	12·18	8,500	1,392	16·4	
						15·6
Site 2.	1.	12·19	10,500	1,930	18·5	
To right of centre.	2.	12·20	12,200	2,360	19·3	
	3.	12·21	13,600	2,340	17·2	
	4.	12·22	13,800	2,520	18·3	
						18·3

Indoor sites (in window sill).		G.M.T.	Illumination in meter candles. In open on Roof Vo.	In window sill Vs.	Daylight Factor. $\frac{Vs.}{Vo.}=\delta$	Average δ
Site 3.	1.	12·23	12,200	2,520	20·7	
Centre of window.	2.	12·24	15,200	2,680	17·6	
	3.	12·25	14,100	2,290	16·2	
	4.	12·26	13,000	2,680	20·6	
	5.	12·27	13,200	2,560	19·7	
	6.	12·28	11,800	1,880	15·9	
						18·45
Site 4.	1.	12·29	11,100	1,610	14·5	
To left of centre.	2.	12·30	9,690	1,410	14·7	
	3.	12·31	10,900	2,110	19·3	
	4.	12·32	12,400	2,170	17·5	
	5.	12·33	15,400	2,660	17·4	
	6.	12·34	14,200	2,310	16·3	
						16·6
Site 5.	1.	12·35	13,600	2,060	15·2	
Extreme left of	2.	12·36	14,800	2,260	15·3	
centre.	3.	12·37	15,600	2,660	17·1	
	4.	12·38	13,300	2,000	15·1	
	5.	12·39	11,400	1,610	14·2	
	6.	12·40	11,300	1,550	13·8	
	7.	12·41	14,900	2,310	15·6	
	8.	12·42	12,400	1,670	13·5	
	9.	12·44	10,800	1,680	15·6	
						15·6

Total average for the four sites is 16·6 for δ.

The Filters used.

The filters selected were twelve in number, and Table II gives the details concerning their transmissions of energy and wave length. The non-selective screens were Wratten filters of certain known reduced intensities. The transmissions (T) for these were :—T=50%, 25%, 12·5%, 6·25% and 3·1% respectively, but unhappily the gelatine films were damaged at the outset of the experiments, and their actual transmissions determined photo-electrically, using a sodium vacuum sensitive cell, were T=41·8%, 19·2%, 9·24%, 3·26% and 1·66% respectively.

For convenience these filters will be referred to as Wratten filters $T=\frac{1}{2}$, $T=\frac{1}{4}$, $T=\frac{1}{8}$, $T=\frac{1}{16}$ and $T=\frac{1}{32}$ respectively. The makers' figures refer, however, to light incident normally whereas the photo-electric measurements refer to light received from the sky as a whole. The latter are necessarily lower than the former on account of the oblique incidence.

Six selective filters were used. Four were of Corning glass of red, orange, green and blue shades, and the actual percentage transmissions for these were determined by Dr. H. H. Poole (Dublin) by means of a Moll thermopile. Figure 2 shows the curves obtained from his data together with the transmission curves of the other coloured filters used.

TABLE II.

To show the wave lengths, light transmissions and relative energy values for the screens and filters used for the culture experiments.

Screen or filter used.	Thickness of screen used. mm.	Wave length in mμ.	Actual percentage light transmitted T.	Average relative energy in arbitrary units (from Dr. Abbot's data).	Total percentage relative energy transmitted E.
Solar spectrum			100	2,880	100
Wratten neutral					
1. Wratten $T=\frac{1}{2}$		400–720	41·8	1,240	41·8
2. ,, $T=\frac{1}{4}$		,,	19·2	570	19·2
3. ,, $T=\frac{1}{8}$		,,	9·5	274	9·5
4. ,, $T=\frac{1}{16}$		,,	3·3	95	3·3
5. ,, $T=\frac{1}{32}$		,,	1·7	49	1·7
6. Ordinary glass		,,	71·0	2,040	71·0
7. Corning red	2.96	620–720	67·1	602	20·9
8. ,, orange	2·96	570–720	73·0	990	34·5
9. ,, green	3·47	490–570	13·4	96	3·3
10. ,, blue	2·96	400–490	60·4	490	17·0
11. Schott and Gen blue	2·21	400–490	50·3	406	14·0
12. Chance blue		400–490	36·2	293	10·2
13. Heat absorbing screen		400–720	27·7	795	27·7
14. Heat absorbing + Corning blue		400–490	16·7	135	4·7

One of these was a blue glass prepared by Schott and Gen of Germany, and the transmission curve was obtained from data supplied by them. The other blue filter was prepared by Chance of Birmingham and the curve for it was obtained by resolving values read from a photograph of the density curve supplied by them. $(\text{Density}=\log\frac{1}{\text{transmission}})$. It may be observed from Figure 2, that the Corning blue transmits a certain low percentage of parasitic red rays. These were eliminated in some experiments, by placing a heat absorbing screen of a green shade over the Corning blue, which effectively cuts off these red rays, but lowers the intensity considerably. A suitable correction has been applied for this, in interpreting the results with the combination of screens. The other blue screens furnish a blue light of a high degree of purity, the Chance blue shows no inclusion of red at all.

Lastly, a filter of ordinary glass was used as a control, and since it was of a greenish tinge the amount of blue light passing through was lowered, and the total intensity of the light received reduced.

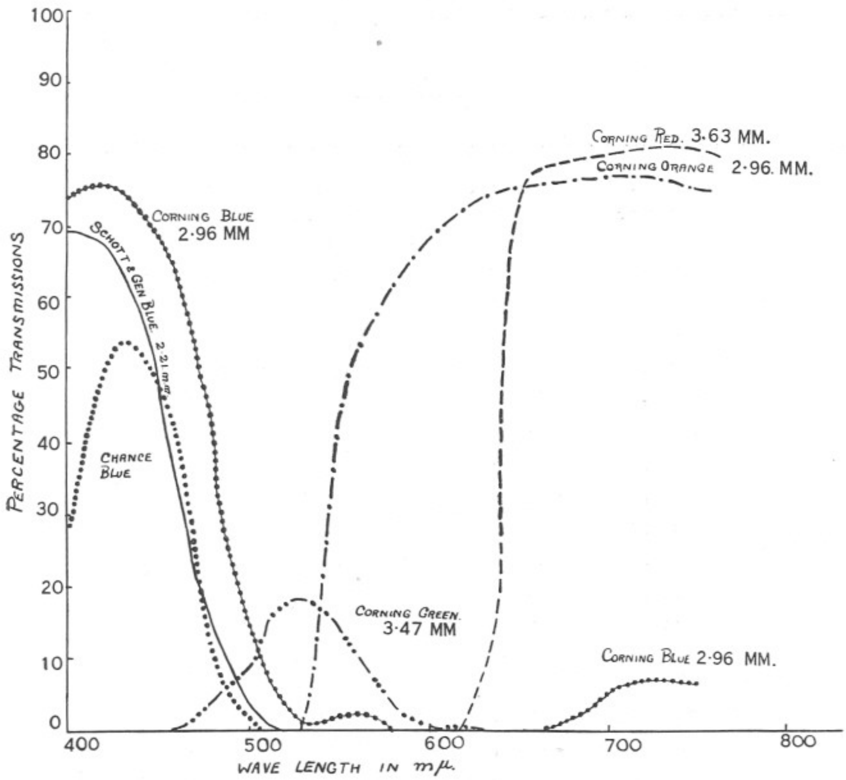

FIG. 2.—The ordinates are percentage transmissions for the selective screens used as determined, using a Moll thermopile. The abscissæ are wave length in mμ. The curves for the Chance blue, Schott and Gen blue glasses were obtained from data supplied by those firms as corrected for reflection losses for light incident normally.

THE RELATIVE ENERGY OF THE FILTERS USED.

Owing to lack of apparatus it was impossible to determine the relative energy transmitted through the screens directly, but it is hoped that this will be done shortly. The energy value is one which is undergoing constant change throughout the day, since it varies considerably in the different portions of the spectrum, and the proportion of the red, yellow, green and blue light changes from hour to hour according to the time of day and the condition of the sky light. An attempt to obtain approximate values of the relative energy in arbitrary units was made in the following manner.

At the Seventh International Congress of Photography, 1929, it was recommended that the following energy values derived from data of Dr. C. G. Abbot (Smithsonian Institute, U.S.A.) should be adopted as defining the spectral composition of mean noon sunlight. Table III shows these values for the visible part of the spectrum.

TABLE III.

RELATIVE ENERGY VALUES FROM DATA OF DR. C. G. ABBOT.

Wave Length.	Relative Energy.	Wave Length.	Relative Energy.
360	16·0	550	101·7
370	20·5	560	100·0
380	25·1	570	98·4
390	30·1	580	97·2
400	45·2	590	95·6
410	57·2	600	95·2
420	65·8	610	94·3
430	69·2	620	93·2
440	77·2	630	92·2
450	86·8	640	91·0
460	92·2	650	89·7
470	96·9	660	88·5
480	99·0	670	86·4
490	100·6	680	84·7
500	101·8	690	82·7
510	101·2	700	80·5
520	101·2	710	78·1
530	101·1	720	76·1
540	100·9		

Using these figures it was calculated that the average value for the relative energy transmitted from 720mμ—400 mμ. was 89·9 units per sq. cm. per minute, and the light transmitted over this region was taken as 100%. The total energy transmitted, E, for the visible spectrum was thus proportional to $89{\cdot}9 \times (720\text{–}400) = 2880$ arbitrary units. Since this is the maximum value for E, it may be taken as 100, and the values of E for the light filters then found by substitution, e.g. considering the Corning red filter $\frac{89{\cdot}9 \times (72-62)}{2880} = 31{\cdot}2\%$, and this is the proportion of red as defined in the spectral curve. Of this the filter transmits 67·1%, so that the total relative energy transmitted is only 21·0%. Column 5, Table II, shows the values of E for all the filters used.

RESULTS OF OBSERVATIONS

Attempts were first made to conduct the experiments in the open, but the temperature was too high for the growth of the diatoms, and the light intensity also, for all but the more heavily screened.

Three series of observations were made indoors :—a preliminary series in May, 1929, followed by a second series June 13th–July 28th, 1929, which is referred to as the "summer" series. In both cases filters 1–10, Table II, were used. During the interval between the second and third series (January–March, 1930), the actual transmissions of the filters had been determined by Dr. H. H. Poole, revealing the presence of parasitic red rays in the Corning blue. In the third set of observations, therefore, precautions were taken to exclude the red rays of the blue filter by placing a heat-absorbing screen over it, as previously described. The light intensity T was then reduced to 15·0 and the relative energy E to 4·7%. The Schott and Gen and Chance blue filters were used in January in addition to the other filters.

Preliminary Series, May 6th–28th, 1929.

Average hours of sunshine 9 hours daily.

Tables IV, V, and VI are the results of observations made upon the rate of growth of the marine diatom *Nitzschia closterium*, when grown in "Miquel sea-water," under selective or non-selective screens as described in the preceding pages. The following list of screens used indicates the order of the cultures in the window, starting from the right-hand side :—

Corning blue		(400 mμ–490 mμ)
,, green		(490 mμ–570 mμ)
,, orange		(570 mμ–720 mμ)
,, red		(620 mμ–720 mμ)
Ordinary glass		(400 mμ–720 mμ)
Wratten filter T.=$\frac{1}{2}$	41·8%	(400 mμ–720 mμ)
,, ,, T.=$\frac{1}{4}$	19·2%	,,
,, ,, T.=$\frac{1}{8}$	9·5%	,,
,, ,, T.=$\frac{1}{16}$	3·3%	,,
,, ,, T.=$\frac{1}{32}$	1·7%	,,

TABLE IV

Sub-cultures of *Nitzschia closterium* Grown in Crucibles under Ordinary Glass, from 6th–28th May, 1929.

Date.	No. of days.	No. of diatoms per unit of volume.
9.5.29	3	18
13.5.29	7	85
16.5.29	10	123
21.5.29	15	270
23.5.29	17	432
28.5.29	22	546

TABLE V.

Sub-cultures of *Nitzschia closterium* Grown in Crucibles under Neutral Wratten Filters from 9th–28th May, 1929.

Date.	No. of days.	No. of diatoms per unit of volume.				
		$T=\frac{1}{32}$	$T=\frac{1}{16}$	$T=\frac{1}{8}$	$T=\frac{1}{4}$	$T=\frac{1}{2}$
13.5.29	4					
16.5.29	7	6	1	42	13	32
21.5.29	12	6	3	60	315	340
23.5.29	14	14	18	218	475	485
28.5.29	19	30	42	590	1400	1206

TABLE VI.

Sub-cultures of *Nitzschia closterium* Grown in Crucibles under Selective Filters of Corning Glass from 13th–23rd May, 1929.

Date.	No. of days.	No. of diatoms per unit of volume.			
		Red.	Orange.	Green.	Blue.
16.5.29	3	2	3	0	2
21.5.29	8	120	69	152	56
23.5.29	10	285	241	270	106

The figures in the tables represent the number of diatoms per unit of volume (0·1 mm.^{3}) as counted with the aid of a hæmacytometer. From these results it would appear during the period of observation for May 1929 :—

1. That the diatoms can utilise light of all wave lengths.

2. That light of reduced intensities is much more favourable than full illumination at this time of year. Light reduced to about 6%–3·3% being the most suitable of the series.

Second Series, June 13th–July 29th, 1929.

Table VII gives the results of observations from a new series of sub-cultures grown under the same conditions as in the preliminary experiments and occupying the same sites as formerly. During this period,

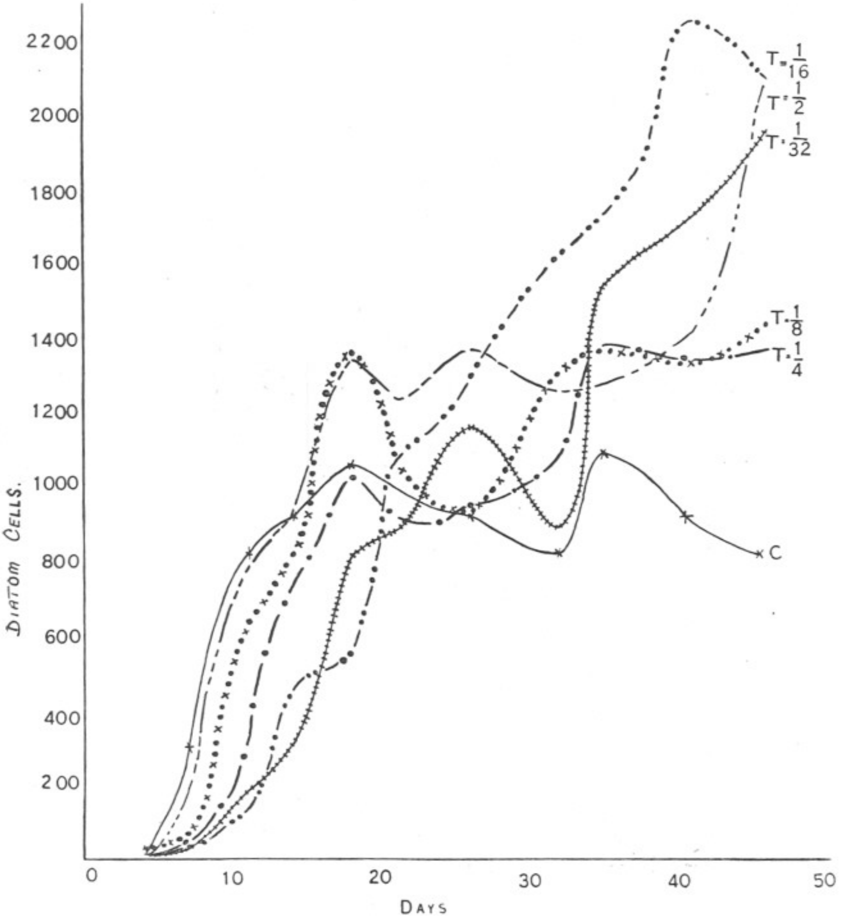

FIG. 3.—The ordinates are numbers of diatom cells in 0·1 cubic millimetre. The abscissæ are days. The curves show the rates of growth of the cultures of *Nitzschia closterium,* when grown under screens reducing the light intensity non-selectively, for a period of 46 days during the months of June and July, 1929.

however, the average number of hours of sunshine was 8 hours 11 minutes for June and 8 hours 49 minutes for July. Many days had 14 hours sunlight. Figures 3 and 4 give the curves obtained from the data.

When grown in ordinary flasks, cultures of *Nitzschia closterium* appear yellowish brown. Distinct colour changes of the cultures grown under the selective filters were observed during this second series, and also throughout the January–March growths. Under the heading of the concluding remarks for the last series, the subject is dealt with more fully. Reference to Figure 6 will show at a glance that a curious drop in the

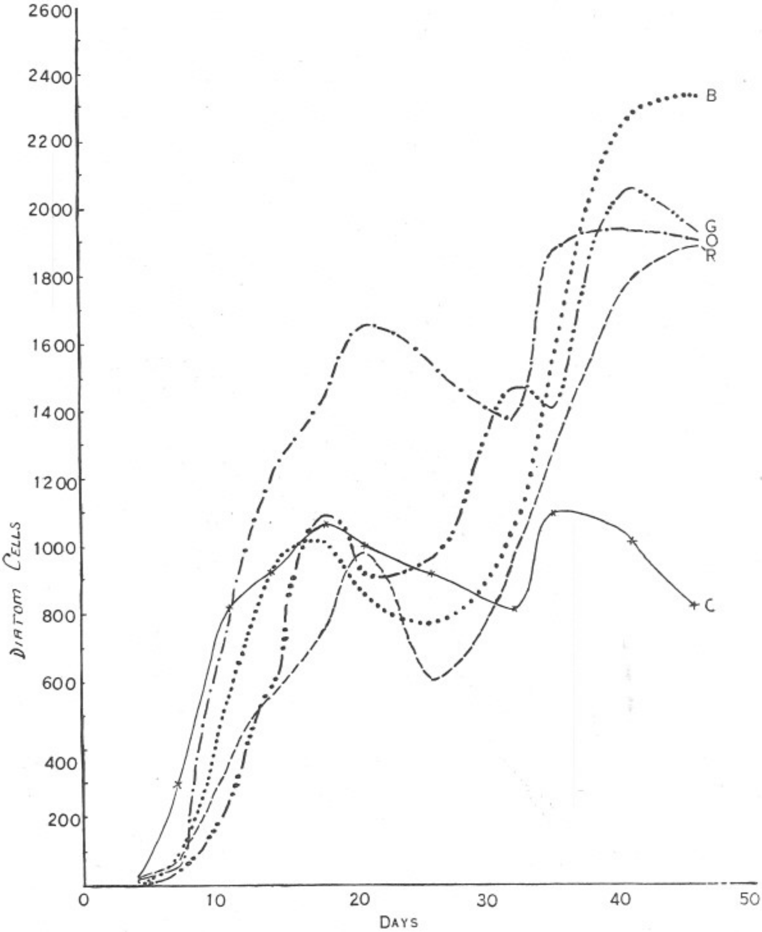

Fig. 4.—The ordinates are numbers of diatom cells in 0·1 cubic millimetre. The abscissæ are days. The curves show the rates of growth of the cultures of *Nitzschia closterium*, when grown under screens reducing the light selectively, for a period of 46 intensity days during June and July, 1929.

number of diatoms is shown by all the growths. Allen and Nelson (1910) state that the siliceous shell of marine diatoms generally, is markedly thinner than in the great majority of other forms, and that this is even more emphasised in cultural forms. Dr. Allen has also stated that it is a common experience for cultures to show periodic decrease in numbers.

TABLE VII.

To Show the Growth of the sub-cultures of *Nitzschia closterium* Grown under Selective and Non-selective Filters, from June 13th–July 29th, 1929.

No. of days.	No. of diatoms per unit of volume.									
	Wratten filters.						Corning filters.			
	$T=\frac{1}{32}$	$T=\frac{1}{16}$	$T=\frac{1}{8}$	$T=\frac{1}{4}$	$T=\frac{1}{2}$	Ordinary Glass.	Red.	Yellow.	Green.	Blue.
4 . .	4	4	24	8	2	15	19	20	9	8
7 . .	22	36	64	147	196	299	68	54	51	92
11 . .	184	132	620	336	792	816	384	786	276	576
14 . .	304	468	816	732	924	920	552	1184	584	920
18 . .	812	544	1360	1024	1344	1060	728	1444	1092	1016
21 . .	884	1072	1060	912	1234	996	976	1652	920	860
26 . .	1160	1296	932	940	1372	920	604	1548	968	772
32 . .	886	1624	1304	1088	1256	816	980	1372	1468	1060
35 . .	1544	1860	1368	1376	1720	1092	1276	1864	1404	1540
41 . .	1728	2256	1330	1340	1412	1012	1788	1928	2064	2272
46 . .	1868	2092	1432	1336	2080	812	1884	1900	1920	2328

Accordingly, a special watch was made for empty frustules, and these were very seldom seen. The problem of the fate of the diatoms, which causes these well-marked falls in the curves, is further discussed in the third series. It may be mentioned here, however, that this decrease can hardly be due to lack of food material. Under the ordinary course of the experiments the volume of the culture solutions was made up to 10 c.c. from time to time by the addition of distilled water, to compensate for the small losses in volume due to evaporation and the minute drops extracted for counting purposes. After the 29th July, 1929, however, the observations were abandoned for about two months. During this time the cultures remained untouched. Yet counts made for all the cultures on September 29th, 1929, showed such dense growths that it was almost impossible to see the ruling on the hæmacytometer when drops were examined in the usual way after the culture solutions had been restored to their original volume of 10 c.c. with distilled water.

Conclusions from Second Series.

Thus it appears that diatoms can utilise light of all wave lengths even in the green region of the spectrum. It is true that the Corning green transmits parasitic rays of yellow and blue, but the percentage is so small as to be scarcely responsible for the astonishing growth under a screen which has a total transmission of light of only 13·5%. Miquel (1892) found that orange light was best suited to the growth of freshwater diatoms. A study of Table VII will show that orange light is highly favourable for growth of the marine diatom Nitzschia, being slightly better than red, which it includes. During the last few days of the experiments the numbers under the blue and green filters exceeded those of the diatoms under both the red and orange. It must be remembered that the Corning blue filter was transmitting a small percentage of parasitic red rays, but results obtained in the third series indicate that they alone are not responsible for the prolific growth under this screen.

The light intensity and energy transmitted by the control (T and E =71%) were too high for good growth, the amounts transmitted by all the reducing non-selective filters being much more favourable. Under the selective filters the cultures grew well, for although the light intensity was in some cases high, e.g. orange and red, the relative energy in these regions is considerably less than for white light (Table II).

Third Series. Winter and Early Spring. January 2nd–April 14th, 1930.

The cultures were set up in the same manner as before, the only difference being in the use of additional filters, viz. the heat-absorbing screen which was placed over the Corning blue, and the extra filters of Schott blue, and Chance blue (Nos. 11, 12, and 13, Table II).

Attention is here drawn to the fact that the transmission curves for 11

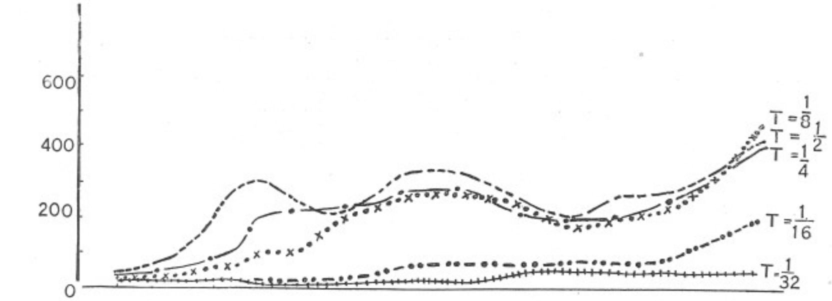

FIG. 5.

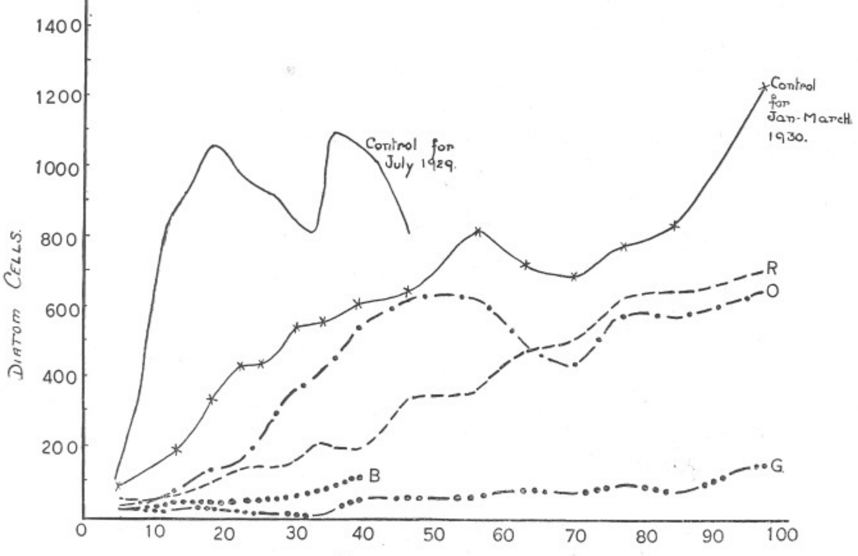

FIG. 6.

FIGS. 5 & 6.—The ordinates are numbers of diatom cells in 0·1 cubic millimetre. The abscissæ are days. The curves show the rates of growth of the cultures of *Nitzschia closterium* when grown under screens reducing the light intensity selectively, Fig. 6, lower portion, and non-selectively Fig. 5, upper portion, for a period of 97 days during January-March, 1930. The curve showing growth under the control screen during June and July, 1929, is also shown.

and 12 were obtained from data supplied by the makers in January, 1930, and it has not been possible as yet to verify these figures, but the absence of any appreciable amount of red has been verified by visual spectroscopic examination.

The average number of hours daily sunshine in January was 1 hour 10 minutes, in February 3 hours 18 minutes, and in March 3 hours 50 minutes. Table VIII and Figures 5, 6, and 7 are records of growth for this period.

Conclusions from Third Series.

1. The results suggest that the total light intensity received is a factor of great importance. Excepting the Schott blue filter, the diatoms under the ordinary glass screen show the best growth, whilst the cultures under

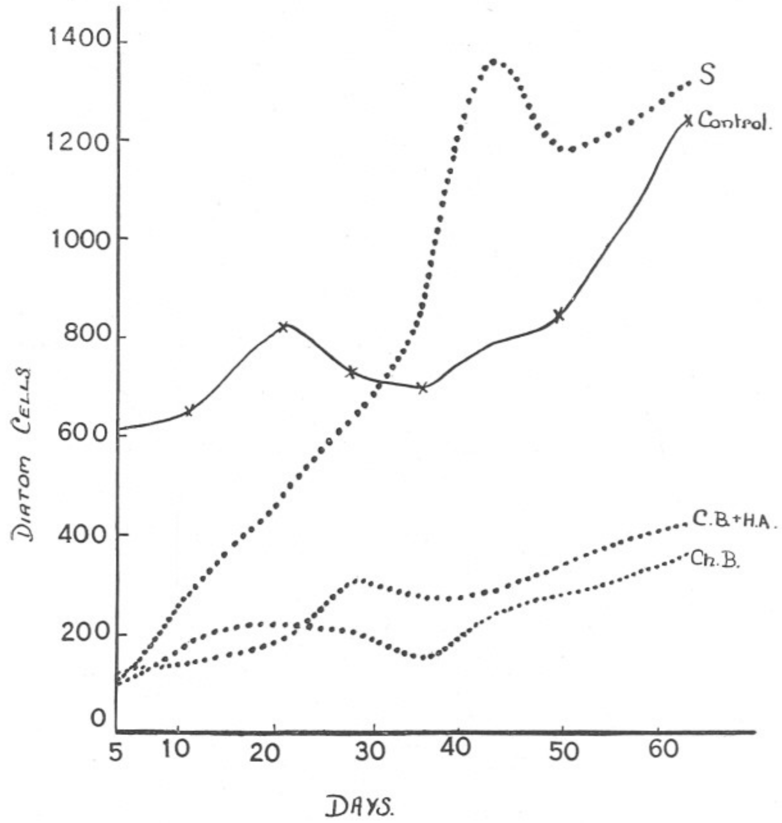

Fig. 7.—The ordinates are numbers of diatom cells in 0·1 cubic millimetre. The abscissæ are days. The curves show the rates of growth of the cultures of *Nitzschia closterium* when grown under the influence of blue light for a period of 63 days during February and March, 1930. The curve showing growth under the control screen during the same period is also given.

the Wratten filters show poor growth where the intensity is heavily reduced. The figures shown in Table IX would suggest that the relative energy is a limiting factor, the ratio $\frac{\text{Relative Energy}}{\text{No. of diatoms}}$ being a constant in the experiments and having a value of 24·5.

TABLE VIII.

To Show the Growth of the sub-cultures of *Nitzschia closterium* grown under Selective and Non-selective Filters from January 2nd–April 9th, 1930, and from February 5th–April 9th, 1930.

Number for diatoms per unit of volume.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
No. of days.	°C.	$T=\frac{1}{2}$	$T=\frac{1}{4}$	$T=\frac{1}{8}$	$T=\frac{1}{16}$	$T=\frac{1}{32}$	Control Glass.	Corning Red.	Corning Orange.	Corning Green.	Corning Blue.	Corning Blue + Heat Ab.	Schott and Gen Blue.	Chance Blue.	No. of days.
—	—	—		—	—	—	—	—	—	—	—				
5	13·1	48	36	32	28	28	88	48	48	32	20				
13	13·2	88	52	32	20	17	188	60	72	20	45				
18	13·1	172	84	56	20	20	332	96	136	26	40				
22	11·8	280	108	64	20	20	432	136	160	16	44				
25	10·6	300	200	96	20	8	432	144	224	9	50				
30	10·1	256	220	98	16	8	544	168	368	8	60				
34	12·6	220	224	152	20	12	560	212	428	8	88				
39	9·9	232	236	216	28	12	616	200	544	57	116	116*	108*	104*	5*
46	10·6	320	272	256	60	40	650	340	624	60	—	140	281	184	12
56	13·3	318	272	260	62	40	825	368	630	60	—	192	484	216	22
63	13·2	250	212	240	64	45	728	488	500	88	—	302	640	200	29
70	14·2	200	200	170	66	40	696	516	436	82	—	276	860	150	36
77	11·2	259	200	200	70	43	788	632	576	100	—	280	1360	232	43
84	12·9	266	240	232	76	40	944	652	584	88	—	332	1180	280	50
97	14·1	408	396	456	156	40	1244	716	656	168	—	420	1316	360	63

* Columns 13–16 give the figures for the cultures started February 4th, 1930.

TABLE IX

To Show that the Ratio of the Relative Energy to the Number of Diatoms tends to be a Constant when the Relative Energy transmitted is Low.

Screen Used.	$T=\frac{1}{32}$	$T=\frac{1}{16}$	Corning Green.	$T=\frac{1}{8}$
Percentage Light Transmitted . . .	1·7	3·3	13·5	9·5
Percentage Relative Energy	1·7	3·3	3·3	9·5
No. of Diatoms at the end of 84 days . .	40	76	88	233
$\frac{\text{Relative Energy}}{\text{No. of Diatoms}}$ . .	23·5	23·1	26·3	25·2
Average Ratio . .	24·5			

2. The large growth under the Schott blue filter ($T=50{\cdot}3$ and $E=14{\cdot}0$) is difficult to account for unless the screen transmits almost the optimum light intensity and energy. This view would explain the relatively poor growths under the Chance blue, and combined Corning and heat-absorbing screens where $T=36$ and $E=10$ for the Chance and $T=16{\cdot}7$ and $E=4{\cdot}7$ for the combination. Under the red filter ($T=67$, $E=30$), and the orange ($T=73$, $E=34$), the rates of growth were very similar to each other, that under the orange being slightly better than that under the red for the greater part of the third series. Unlike the summer results, the growths under these two filters were poorer than that under the ordinary glass screen. Under the green filter ($T=13{\cdot}5$, $E=3{\cdot}3$) growth was poor, and suggested the importance of the relative energy transmitted when the light intensity was low (Table IX).

3. The difference in colour exhibited by some of the cultures has already been referred to. When grown in ordinary culture flasks the growths are yellowish brown. Under the coloured screens they vary from a rich dark brown shade to decided greenish tints. Under the reduced light of the non-selective Wratten filters the cultures remained yellowish brown as under the control. These colours are not due to differences in the numbers of diatoms present, for counts made on April 9th gave 1244 per unit of volume under the glass filter and 1316 under the Schott blue, yet the culture under the latter was the rich brown colour, whilst under the ordinary glass the culture was yellowish brown. Under the red and yellow filters the cultures always assumed a decided yellow-green shade, whilst under the blue and green they were decidedly dark brown, i.e. the cultures tended to assume colours complementary to those in which they were grown. The dark brown colour of the cultures under the blue and

green filters is probably an adaptation which helps to compensate the deficiency of red and yellow rays by furnishing an added power to absorb the rays available. This is of interest when it is considered that marine diatoms are frequently abundant between depths of 5–15 metres in the sea, being seldom found very near the surface because of the harmful effect of high light intensity. It is known that at a depth of 5 metres most of the red rays are absorbed, whilst at 15 metres the light that penetrates is reduced to about 10–20% of the total intensity and is deprived of all the red, practically all the yellow, and consists of green and blue light only.

4. The cultures showed the same periodic decrease in numbers of diatoms similar to those of the summer series. Drops of solution from the cultures gave pH values from pH 8·8–9·2, showing that the solutions in which the diatoms grew were strongly alkaline. Whilst silica is highly resistant to acids it yields to alkalines, and it is suggested that the falling off in numbers is due to the fact that the thin shells of the dead frustules are dissolved in the culture solutions. This view is upheld by the results of the following experiments. A certain volume of culture solution containing a known number of diatoms per $mm.^3$ was gently heated and kept just below boiling point for some time. The solution was then made up to the original volume with water and counts made for the number of individuals present in the usual way. Nearly all the diatoms had disappeared, suggesting that all but the most resistant frustules had dissolved, the boiling being a quick method of showing what would be a slow process in the culture solutions.

GENERAL CONCLUSIONS.

Three series of cultures of the marine diatom *Nitzschia closterium* were grown under selective and non-selective filters, the transmissions of which were known between definite limits of wave length.

From results of observations on the growth of the cultures it would appear :—

1. That the amount of energy transmitted is of greater importance than the precise wave lengths between which the energy so transmitted lies. When the amount of radiation is small then growth tends to be proportional to the relative energy, but when the energy received is too intense then the effect is harmful to the cultures.

2. At all times the cultures tend to show chromatic adaptation when grown under selective filters, assuming colours which are complementary to those in which they are growing, e.g. a dark rich brown shade under the green and blue screens and a decided green colour under the red and yellow.

Since these experiments were completed, Klugh (1930) has published a further report on the effects of light of different wave lengths, but of equal intensities, on the photosynthetic rates for certain green and red algæ. His results would appear to confirm the conception of the complementary nature of the brown colour.

3. The cultures are subject to periodic decrease in numbers, which is probably due to the thin siliceous shells of the diatoms being dissolved in the culture solutions which have become highly alkaline.

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