OXYGEN PRODUCTION BY THE DIATOM *COSCINODISCUS EXCENTRICUS* EHR. IN RELATION TO SUBMARINE ILLUMINATION IN THE ENGLISH CHANNEL

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(Text-figs. 1–9)

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Introduction

Much attention has been directed in recent years to the analysis of the productivity of the sea. One of the most fundamental processes to be considered is the photosynthesis of marine plants, whereby the inorganic is converted to the organic by means of the energy in daylight.

Until the autumn of 1932 the measurement of photosynthesis and of light in the open sea had apparently only been measured independently, the former notably by Marshall & Orr (1928, p. 321), and the latter by Poole & Atkins (1926, p. 177, etc.).

The correlation of the two kinds of measurements appeared to be the next step; and, since the measurement of light is a more rapid process than that of photosynthesis, it was hoped that the establishment of any simple correlation might lead to more rapid means of assessing the possible productivity of natural waters. Measurements of photosynthesis were accordingly made by Marshall & Orr's method, with the co-operation of Dr W. R. G. Atkins, F.R.S., for the simultaneous measurements of light.

In 1934 a preliminary report by Pettersson, Höglund & Landberg (1934, p. 3), showed that they had undertaken a project rather similar to mine at almost the same time. Their results form a most interesting counterpart to mine, for in both cases the illumination was measured as well as the photosynthesis; but my aim was to expose diatoms at a set of constant depths and measure the variations in the illumination, while theirs was to expose mixed plankton samples to a constant illumination by varying the depth of suspension in such a way as to counterbalance the changes in daylight. Their method is ingenious but makes no allowance for the inevitable variations in the spectral composition of light that occur with change in depth of the water. Moreover, their plankton (Pettersson *et al.* 1934, p. 9) consisted of a mixture of different species of plants and even some animals, so that their results are more difficult to interpret than those obtained by using a pure culture of diatoms. This difficulty is also apparent in the methods adopted by most previous workers, except Marshall & Orr, for estimating photosynthesis (e.g. Gaarder & Gran, 1927, p. 8; Steemann Nielsen, 1932, p. 5, etc.). It was therefore considered a sufficient reason for spending much time and labour in growing cultures of the diatom *Coscinodiscus excentricus* Ehr. for use in the present investigation.

The oxygen production of these cultures was measured as an index of their photosynthesis, in preference to the carbon dioxide consumption, simply because of the ease with which oxygen in sea water may be measured by the Winkler method.

All the measurements of light, both in air and in the sea, were made for me by Dr Atkins, to whom, in conjunction with Dr H. H. Poole, I am also indebted for the following method of computing the energy in submarine illumination.

METHOD OF COMPUTING THE ENERGY IN SUBMARINE ILLUMINATION

The first difficulty in measuring submarine illumination is introduced by the changing spectral composition of the light that accompanies increasing depth below the surface. This is due to the differential absorption, or rather extinction, of light of different wave-lengths by the water. In air, measurements of the change in intensity of the light in one region of the spectrum are closely proportional to changes in intensity in the whole spectrum: under water, the proportionality is rapidly lost as the depth increases, because the intensity in each part of the spectrum decreases at an independent rate. Thus in clear water, for example, the light becomes not only fainter but much bluer, as it descends.

It is apparent that it is not correct to express submarine illumination in terms of the "lux" or metre-candle, nor is it convenient as the visual scale is not necessarily the same as the photo-electric, and the differential extinction of the spectrum under water alters the ratio of the one scale to the other.

The illumination in different submarine situations can therefore only be satisfactorily expressed and compared in terms of energy, if the comparison is to be valid, irrespective of the wave-lengths of the radiation.

Neither light nor energy, however, could be measured directly under water; but the work of Atkins & Poole (1936 b, p. 1), on the luminous efficiency of daylight, and the standardization of photo-electric cells for the measurement of energy (Poole & Atkins, 1936, p. 363), allowed the energy to be computed indirectly, with a reasonable degree of accuracy.

Since the illumination to be dealt with is all submarine, it is possible to limit the radiant energy, which need be measured, to that within the visible spectrum (3800–7200 A.). The energy in the ultra-violet (wave-lengths shorter than 3800) is so rapidly absorbed by the sea, and forms, in any case, so small a proportion of the total energy in daylight, that its effect upon photosynthesis may be neglected (Spoehr, 1926, p. 117); while the energy in the deep red and infra-red (wave-lengths longer than 7200) is also absorbed very rapidly, and is so unlikely to effect any photosynthesis directly, that it may also be neglected. Moreover, any inaccuracy due to these assumptions would only affect the uppermost layers of the sea, where it is in any case difficult to obtain accurate measurements of either light or photosynthesis under ordinary sea-going conditions in the Channel.

Within the visible spectrum it seems probable that diatoms may make use of energy for photosynthesis almost equally in radiation of all wave-lengths (Stanbury, 1931, p. 651). This appears to hold good in spite of the heavier absorption shown by chlorophyll in certain restricted bands of the spectrum, particularly in the red region; it is possible that the accessory pigments in the diatoms may be responsible for absorbing the energy in other regions of the spectrum (p. 332).

It is therefore assumed, as a working hypothesis for the present research, that the total energy in all wave-lengths within the visible spectrum gives the best measure of the energy available for diatom photosynthesis in the sea.

Table I shows the method of computing the total energy at a series of depths, from the data obtained on August 28 1933. Values for light of different wave-lengths and the total energy on August 4 1933, are shown graphically in Fig. 1.
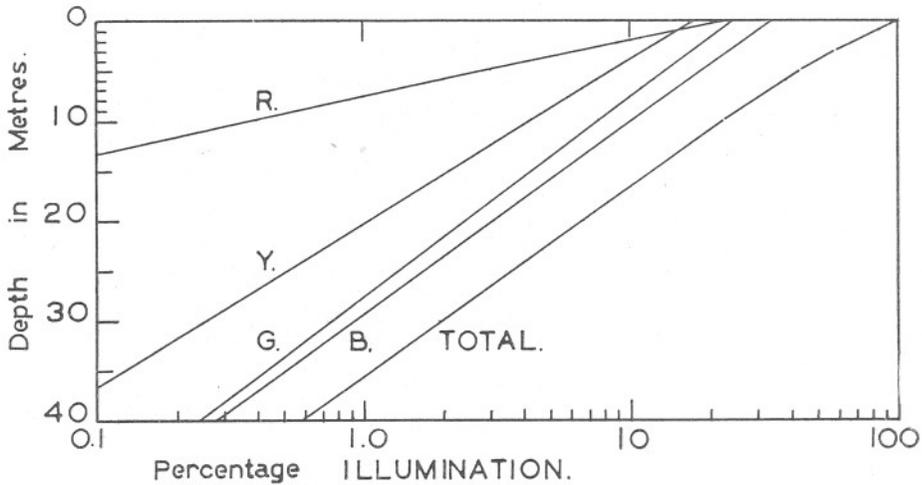
Fig. 1. Submarine illumination on August 4 1933. The percentages are given on a logarithmic scale. If 100 % represents the subsurface daylight, the relative distribution of energy in air between "blue" 3800–4900 A., "green" 4900–5600 A., "yellow" 5600–6200 A., and "red" 6200–7200 A., is shown by the respective starting points of the lines B, G, Y, and R, at the surface (cf. Table II, column 5). The slope of these lines is determined by the extinction coefficients. The curve on the extreme right shows the total illumination percentages.

The computation is made in a series of steps:

(i) Measurement of the Illumination in Air

The intensity of light in air is measured photo-electrically and expressed in lux.

The amount of light falling on a horizontal surface in air in a given time is the product of the intensity and the time; it has been called the "vertical illumination integral" (Atkins & Poole, 1936a, p. 257), and is measured in kilolux-hours. To allow for the varying intensity of daylight from hour to hour, and even from minute to minute, the intensity is recorded automatically every minute on a graph; the illumination integral is then obtained from the area enclosed on the graph, by the curve for intensity values and the time axis.

(ii) *Measurement of the Illumination in Water*

When light passes into water, the rays of different wave-length are extinguished at varying rates, owing to differential absorption, scattering, and other effects. Ideally, therefore, the rate of extinction for each wave-length should be measured separately: in practice a satisfactory degree of uniformity is achieved by measuring the rates for wave-lengths in four consecutive groups, namely "blue" 3800–4900 A., "green" 4900–5600 A., "yellow" 5600–6200 A. (measured by difference) and "red" 6200–7200 A. (B, G, Y, and R, Fig. 1). Colour filters were combined with photo-electric cells to measure the intensity of submarine light within each of the spectral regions, at a series of depths (Atkins & Poole, 1933, p. 134). The results were expressed as percentages of the intensity of subsurface light in the same spectral region (illumination percentages Table I and Fig. 1).

In water of uniform transparency, these illumination percentages decrease exponentially with depth, so that the rate of decrease, or extinction, can be expressed as a coefficient (extinction coefficients, Table I), which can then be used to calculate the percentages at intermediate depths.

TABLE I. COMPUTATION OF ENERGY IN SUBMARINE ILLUMINATION, ON AUGUST 28 1933

Exposure from 1140 to 1650 G.M.T. = 5·17 hr.
Vertical illumination integral in air = 201 kl.-hr.
Subsurface illumination (subtracting 15 % for reflexion) = 171 kl.-hr.
Derived subsurface energy integral = 256·5 joules or 61·6 g.-cal.
Factors for weighted energy percentage as for "sun and cloud" (see Table II, column 3).
Vertical extinction coefficients, blue 0·14, green 0·13, yellow 0·16, red 0·46, uniformly down to 30 m. (see Table III, p. 308).

Depth in m. ...	0		1		5		10		20		30	
Blue:												
Illumination %	100	—	86·9	—	49·5	—	24·4	—	6·1	—	1·5	—
Weighted energy %	—	26·7	—	23·2	—	13·2	—	6·5	—	1·6	—	0·4
Green:												
Illumination %	100	—	87·8	—	52·2	—	27·3	—	7·4	—	2·0	—
Weighted energy %	—	24·2	—	21·3	—	12·6	—	6·7	—	1·8	—	0·5
Yellow:												
Illumination %	100	—	85·2	—	44·9	—	20·2	—	4·1	—	0·8	—
Weighted energy %	—	19·7	—	16·8	—	8·8	—	4·0	—	0·8	—	0·15
Red:												
Illumination %	100	—	63·0	—	9·9	—	1·0	—	0·0	—	0·0	—
Weighted energy %	—	29·4	—	18·5	—	2·9	—	0·3	—	0·0	—	0·0
Total Weighted energy %	—	100·0	—	79·8	—	37·6	—	17·5	—	4·2	—	1·05
Energy Integral in Joules	—	256·5	—	204·6	—	96·5	—	44·8	—	10·8	—	2·7

(iii) *Assessment of Energy in the Illumination*

To convert the measurements of illumination into terms of energy it is necessary to know two more factors: the total amount of energy within the visible spectrum, represented by the illumination integral, for the daylight in air; and the distribution of that energy between the four spectral regions.

The distribution is derived from Abbot's curve (quoted by the International Congress of Photography, 1929, p. 152) for the relative energy distribution in mean-noon sunlight (Table II, column 3). These values were modified on most days, to allow for sky light being bluer than sunlight, the factors for weighting being derived from values given by Walsh (1928, p. 88), and Atkins & Poole (1931 a, p. 34), as shown in Table II, column 4. The propor-

TABLE II. RELATIVE ENERGY DISTRIBUTION IN THE SPECTRUM

1 Spectral region	2 Range in A.	3 % relative energy in mean-noon sunlight (Abbot)*	4 % relative energy in blue sky light (Walsh; Atkins & Poole)	5 % relative energy in daylight (sun 2 + sky 1)†
Violet-blue	3800–4900	26·7	50	34·5
Green	4900–5600	24·2	24·3	24·2
Yellow	5600–6200	19·7	12·3	17·2
Red	6200–7200	29·4	13·4	24·1
		100	100	100

* Taken as the % relative energy in light from sun and cloud with no blue sky on August 28 1933.
† Taken as the % relative energy in mixed daylight during all other exposures in 1933 and 1934.

tion of sunlight to sky light on the days of the experiments was usually taken as two to one, on the basis of Atkins & Poole's (1936 a, p. 260) determinations; this gave the relative energy distribution shown in Table II, column 5 and applies to Fig. 1. Once, on August 28 1933, when the daylight was a mixture of bright sunlight and of light reflected from white clouds with no clear blue sky, the distribution of energy was taken as equivalent to that in mean-noon sunlight (Table II, column 3); this is the instance shown in Table I.

The total energy in air is derived from the illumination integral by taking the energy, within the visible spectrum, as 1·5 joules or 0·36 g.-cal. in 1 kl.-hr. per cm.^{2},* when the source of light is mean-noon sunlight.

The same conversion figure was also used for daylight, although only a

* *Conversion of kilolux-hours to joules.* The flux of light in 1 kl. (1000 metre-candles) on 1 cm.^{2} is 0·1 lumen. If it be assumed that the luminous efficiency of daylight (3800–7200 A.) be 240 lumens per W. (= 1 joule or 10^7 ergs per sec.), the energy flux in 0·1 lumen is 0·1/240 or 0·00417 joules per sec. If the flux be maintained for 1 hr. (1 kl.-hr. per cm.^{2}), the amount of energy transmitted will be 0·00417 × 3600, or 1·5 joules, or 0·36 g.-cal., taking the standard value of 4·183 joules as equal to 1 (15° C.) g.-cal. Poole & Atkins (1936, p. 377), give 269 lumens per W. as the luminous efficiency of mean-noon sunlight (4000–7600 A.). This has been modified to 240 lumens per W., for the present range of 3800–7200 A., on Poole & Atkins' suggestion, in view of the lower value of 223 lumens per W. found by them for Abbot's mean-noon sunlight (4000–7600 A.) by calculation.

portion of the energy comes from sunlight and the rest from the sky. This is possible, if a Burt sodium cell is used to measure the illumination integral, the constants for sunlight and sunlight with sky light being not very different for this type of cell, so that they may be taken as equal without serious error.

To sum up, if we know the extinction coefficients for four adjacent spectral regions in sea water, the relative energy distribution between the four regions in air and the total energy represented by the illumination integral in air, it is possible to calculate the total radiant energy in the visible spectrum at any depth in the sea, during a given time. This is here called the energy integral, and expressed either in joules or gram-calories per square centimetre (Table I).

One other correction has been introduced, and that is the subtraction of a rather arbitrary amount from the illumination for the loss of light by reflexion from the surface of the sea (Table III, p. 308).

In Fig. 1, the "weighted energy percentages" for August 4 are plotted on a logarithmic scale against the depth on a plain scale; the figure shows that the colour of the illumination changes progressively with depth below the surface of the sea, since the slope of the straight line for each colour is determined by its extinction coefficient, and is different in each case. The particular case illustrated is typical of clear water in the English Channel, where red light is the most rapidly extinguished, green has the greatest penetrating power, blue follows closely on green, and yellow is intermediate between blue and red. The figure also shows the relative distribution of energy between the four spectral regions, blue, for instance, having the greatest share in air, but gradually losing its preponderance over green with increasing depth under water.

Although this change in colour of light at different depths in the sea is striking, its possible effect upon photosynthesis is outside the scope of the present work, in which the energy integral is assessed irrespective of the wave-lengths and the spectrum has only been subdivided for the purposes of making the assessment.

Apparatus and Methods for Measuring Illumination

Standards

All the photo-electric cells used for the present work were standardized for the measurement of visible light as described by Poole & Atkins (1935, p. 1). The calibration is in metre-candles, against a carbon arc (1935, p. 11) and "artificial mean-noon sunlight" (1935, p. 16). The two scales agree well in bright mixed daylight.

Illumination Integral

The vertical illumination integral (p. 304) is measured by means of a Burt sodium cell, mounted with its opal plate set horizontally on the roof of the Plymouth Laboratory (Atkins & Poole, 1930, p. 305; 1931*b*, p. 617).

This cell, when used in daylight, has its maximum sensitivity in the violet

TABLE III. DETAILS OF EXPERIMENTAL EXPOSURES, AND DATA USED IN CALCULATING SUBMARINE ENERGY

Station ...	Eddystone Lighthouse, depth 60 m.					S. of Stoke Point, Shagstone bearing N. by W. depth 53 m.
	Bearing S.E.	Bearing N.W.	Bearing N.W.	Bearing S.E.	Bearing S.E.	
Date ...	July 25 1933	Aug. 4 1933	Aug. 28 1933	July 12–13 1934	July 19–20 1934	Aug. 9–10 1934
Time of start, G.M.T.	0945	0910	1140	2120	2035	*A*, 0630 *B*, 1032 *C*, 1434 *D*, 1835
Duration in hours ...	6·25	5·58	5·17	23·7	22·7	*A*, 4 *B*, 4 *C*, 4 *D*, 11·5 } = 23·5
Sea	Flat calm	Calm, with slight ripple	Moderate swell, rising	Fairly calm, rising to swell on 13th	Slight swell, rising considerably on 20th	Moderate, rising during 9th to heavy swell on 10th
Wind	None	Light E.	Light	Light S.W.	Fresh S.E., rising	Light N.W., veering to S.W. and rising
Sky	Overcast, clear after 1300	Clear, with brilliant sun	Overcast throughout	Sun and cloud, some rain on 13th	Sun to overcast	Sun, soon overcast
Illumination integral in kl.-hr.*	338	197	171	408	401	*A*, 108 *B*, 152 *C*, 93 *D*, 6·7
Secchi disk, average	12 m.	14 m.	13 m.	—	14 m.	11·5 m.
Extinction coefficients for:						
"Blue"	0·14†	0·12†	0·14	0·082	0·082	0·142
"Green"	0·13†	0·115†	0·13†	0·095	0·095	0·105
"Yellow"	0·16†	0·14†	0·16†	0·093	0·093	0·13
"Red"	0·48†	0·414†	0·46†	{ 1–5 m. = 0·358 5–10 m. = 0·250 10–30 m. = 0·203	{ 0·358 0·250 0·203	{ 1–5 m. = 0·456 5–35 m. = 0·281

* Corrected for surface loss (p. 310).
† Derived from Secchi disk readings (p. 309).

region of the spectrum: nevertheless, it gives a "tolerably correct measure of the daylight" (Atkins & Poole, 1936a, p. 253, fig. 2). Thus, on a fine summer day, the recorded variations in intensity of violet light can be taken as indicative of the variations in intensity of daylight as a whole, at least between the hours of 0500 and 1900 G.M.T., when the colour of the daylight does not alter materially. All the short exposures fell between these hours; the 24 hr. exposures naturally included the hours before 0500 and after 1900, when the colour of daylight does alter quite appreciably, but during these hours the intensity is so low that the error introduced by estimating the total light from a measure of the violet light is scarcely significant.

It has been assumed that the illumination integral, measured on the roof of the Laboratory on the shore of Plymouth Sound, may be taken as a fair integration of the illumination during the same period at the surface of the sea about 10 miles to the south at the stations where the experiments were carried out. Uneven distribution of cloud over land and sea may sometimes have invalidated this assumption to some, but probably not to any very great, extent (p. 311).

Extinction Coefficients*

The coefficients (p. 305) were not always determined in the same way. In 1933 Dr Atkins was prevented by illness from carrying out the full programme of photo-electric measurements; the extinction coefficient for blue light had therefore to be estimated from Secchi disk readings, on July 25 and August 4, but on August 28 Dr Atkins determined it directly with a gas-filled potassium photo-electric cell (Poole & Atkins, 1928, fig. 2, p. 466). The coefficients for green, yellow, and red for each of these days were derived from those for blue, by reference to the complete data obtained for four regions at the same station in the previous year (Atkins & Poole, 1933, table IV, pp. 150 *et seq.*).

In 1934 the extinction coefficients were measured by means of the Bergmann selenium rectifier cell (Poole & Atkins, 1933, pp. 538 *et seq.* and 1934, pp. 727–36), after correcting for the curvature of the relation between the current and illumination. On July 12 the coefficients for blue, green, and red were measured directly, leaving only yellow to be derived from the "yellow/blue ratio". On July 19 the Secchi disk reading showed a degree of transparency similar to that of the previous week, and the same coefficients have therefore been used for both days. On August 9, all four extinction coefficients were determined photo-electrically (Table III).

The Secchi disk readings, used for estimating the coefficients when no photo-electric measurements were available, were taken from the deck of S.S. *Salpa* and were strictly comparable with those taken in previous years (Poole & Atkins, 1929, p. 309), during the establishment of the empirical formula

$$\mu_{v_{0-20}} = 1 \cdot 7 / D,$$

* Pettersson (1934, p. 7); "absorption coefficient" of Poole & Atkins' earlier papers; and "vertical transmissive exponent" of Clarke (1933, p. 317).

where μ_v is the vertical extinction coefficient for blue light, and D the maximum depth of visibility of the Secchi disk. Such a formula can only give a rough approximation, with no evidence as to the opacity of different water layers; but several series of measurements, made in the open water of the English Channel in the summer of previous years, seemed to show that there is very little change in opacity from depth to depth, and even from day to day, in the absence of heavy gales. Since the weather in July 1933 was exceptionally fine the coefficients derived from the Secchi disk readings may be taken as reasonably reliable.

Surface Loss

Atkins & Poole (1933, p. 148) give the loss of light by reflexion from the surface of the sea as varying between 2 %, when measured during a glassy calm, through 5–17 % for light winds, up to 25 % for moderate winds; beyond this it is not practicable to determine the loss directly.

No direct measurements of the surface loss were made during any of the present exposures, but an arbitrary loss of 15 % has been assumed for all the exposures, except the last, in spite of certain differences in the conditions at the sea surface (Table III).

For the last series of exposures on August 9–10 1934, the energy values have been plotted in Fig. 6 as though there had been no surface loss. This brought the curves for periods A (0630–1028 G.M.T.), and B (1032–1430) into the same relation with the points for oxygen production as in the previous year (Figs. 3–5); but left a discrepancy in the case of period C (1434–1828). By subtracting a surface loss of 33 % from the value for C, the curve $C \times 0.66$ (Fig. 6) was obtained and agreed more nearly with the oxygen-production points for the same period. So low a value for C was independently suggested by the evidence of a photographic light-recording apparatus exposed at sea during the experiment. This apparatus (constructed by Dr L. E. Bayliss) floated alongside the surface cage of bottles, with its window just awash, and gave a series of values for the illumination integrals, in which C was about 30 % lower than the value obtained by the photo-electric recorder on the roof of the Laboratory at Plymouth. If these photographically determined values (kindly supplied to me by Dr Bayliss from unpublished data) are given in relation to an illumination integral of 108 kl.-hr. for period A, so as to be directly comparable with the photo-electrically determined values, we get the following figures:

August 9–10	Period A	B	C	D	
Kilolux-hours	93	130	52	5·5	Photographically determined at sea (Bayliss)
	108	151	60	6·4	The same, adjusted to 108 for period A
	108	152	93	6·7	Photo-electrically determined on shore

Considering the close concordance for periods *B* and *D*, after adjustment of *A*, the difference in the values for *C* is definitely significant. It may have been due to an uneven distribution of cloud reducing the light more at sea than at the laboratory; or it may have been due to the sea's being rougher during period *C* than earlier in the day (Table III) and causing greater reflexion loss at the surface, or to a combination of these factors.

With regard to surface loss, during periods *A* and *B*, it is suggestive that the subsurface illumination integral determined photographically at sea is just about 15 %* lower than that determined photo-electrically in air (93–108). This agrees with the usual 15 % allowed for surface loss during all the other exposures. The fact, therefore, that the energy values appear to fit the oxygen values for periods *A*, *B*, and *D*, without allowing for this loss, probably means that the diatoms were producing more oxygen per million cells for a given amount of energy than those of the previous year. This would have been quite possible if the cells had been larger or more active in 1934 but cannot be proved, as neither the size nor the activity of the cells was measured.

MATERIAL AND METHODS FOR MEASURING PHOTOSYNTHESIS

Choice of Diatoms

Biddulphia regia M. Schultze and *Coscinodiscus excentricus* Ehr. were both used for preliminary experiments, but the cells of the former were found to be too large and heavy to remain evenly distributed in the samples, or to give consistent results. *C. excentricus* was, therefore, used in all the experiments recorded below. It is an oceanic species of world-wide distribution; but in the English Channel it occurs most commonly in winter and early spring. The cells are disk-shaped and very regular in size, large enough to be counted easily or picked out with a pipette under a low-power binocular microscope, and less liable to damage than more irregular or spiny forms. Dr Lebour (1929, p. 36) gives the diameter of the cells as 50–90 μ, but it was noticed that the cells, in culture, decreased to about 40 μ as the summer progressed. They formed chains of two, four, six, or even eight loosely associated cells when the cultures were healthy, but these could be easily broken up by shaking in order to distribute the individual cells evenly in the samples.

Growth of Cultures

All the cultures were started from single cells from the early spring plankton, and were maintained in the laboratory for the following 6 months by making

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* When the photographic and photo-electric instruments were exposed side by side on the Laboratory roof they agreed to within 5 %, giving respectively 505 and 480 kl.-hr. for the illumination integral on July 12–13 1934. The agreement may not be so close under water, but the 15 % difference shown on August 9 is probably of the right order.

subcultures every 10–15 days. The cultures were grown in Allen & Nelson's (1910, pp. 427–8) "Miquel Sea Water" made up in "Berkefeld" filtered (*ibid*. pp. 432–3) "Outside Water", from the English Channel, with the addition of 1 ml. per l. normal $NaHCO_3$ and 3 mg. per l. SiO_2 (in the form of sodium silicate). Allen & Nelson (1910, p. 445) had shown silicates to be of value in increasing the uniformity of the cells of *C. excentricus*; and, although the amount of added silicate was not great,* control cultures showed that it was sufficient to improve the growth.

To reduce the bacterial content of the medium it was brought just to the boil (not to 70° C.) after addition of the nutrient salts; this precipitated much of the silicate with the ferric phosphate, leaving nitrates rather in excess. Dr L. H. N. Cooper, who kindly made some special analyses for me, found that the freshly prepared and boiled Miquel Sea Water contained about 2·5 mg. per l. P_2O_5 in solution, or about one-quarter of the amount added, and about 0·007 mg. per l. Fe. Culture medium, in which growth of diatoms had nearly come to a standstill, contained 2·25 mg. per l. P_2O_5, and at most 0·002 mg. per l. Fe (see Appendix, p. 343).

If this medium was kept in a covered vessel, and not inoculated, it remained quite clear for several months.

All the glass vessels used for cultures and experiments were sterilized by dry heat.

The stock subcultures were kept in 125 ml. flasks, containing 50–60 ml. of the medium, and closed by an inverted glass dish; the large quantities of culture required for the photosynthesis experiments were grown in 3 l. flasks placed in the north windows of the aquarium and screened by butter muslin to reduce the light intensity; four or five of these large flasks, if fairly heavily seeded, produced a sufficiently thick crop of actively growing diatoms for an experiment in about a week or 10 days.

The temperature in the aquarium ranged from about 15 to 19° C., with a short period in August 1933 when it rose to a maximum of 21° C. and stopped the growth of the cultures.

Sterility of the Cultures

Sterility from bacteria is difficult to attain in diatom cultures and cannot be claimed here with certainty, since no bacterial counts were made. It is also arguable that, although Dr Allen's unpublished plating experiments with similar cultures gave negative results, bacteria, which would not grow on agar, may yet have been present in the sea-water medium. On the other hand, the diatom cultures were apparently highly sensitive to bacterial infection and could only be grown freely after repeated washing in sterile culture fluid

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* Atkins (1923, p. 156) showed that sea water, after boiling for 3 hr. in glass, may contain as much as 5·5 mg. per l. SiO_2. The heating, in the present case, is insufficient to have this effect.

and treatment with iodine.* The resultant cultures showed none of the clouding characteristic of heavy bacterial infections, but remained crystal clear when left for 5 weeks or more.

It was therefore assumed that the cultures were at least free from serious bacterial infection.

Exposure of the Diatoms in the Sea

After a number of preliminary experiments, photosynthesis in the sea was estimated by the following elaboration of Marshall & Orr's method (1928, fig. 1, pp. 321 *et seq.*).

Six bottles were exposed at each depth chosen for investigation: three round 180 ml., clear-glass, reagent bottles (*R*, Fig. 2) exposed to light from all sides; two slightly smaller, rectangular, medicine bottles (*F*) blackened on all sides but one, which faced upwards to receive only light falling on a horizontal surface; and one round bottle (*B*) enclosed in a double black-cloth bag and thereby protected from all light. The "flat" medicine bottles were included for comparison with the photo-electric cells which are also affected only by light falling on a horizontal surface.

All the bottles were sterilized and filled from a uniform mixture of about 15 l. of diatom culture and an equal volume of fresh Miquel medium. Cultures of the same age and strain were always selected for any one experiment. In 1933 the culture was mixed by hand in two sterilized earthenware jars; in 1934 it was mixed and aerated for 3 hr. by compressed air led in through a distilled water trap and a cotton-wool plug. The bottles were filled with the mixture, by means of a glass siphon, in a dim light;† they were then kept in the dark until they were lowered into the sea, except for a short exposure to dim light while being tied into galvanized iron wire cages (Fig. 2) in the ship's cabin.

At the beginning of all experiments, and also at the end of long experiments, 2 or 5 ml. samples of the culture were fixed with formalin so that the diatoms might be counted at leisure.

The wire cages were each slung by cords from all four corners, so as to hang horizontally. They were attached to a buoyed cable, 155 m. long and weighing 22 kg., anchored in 55–65 m. of water. Cages below 5 m. from the surface were hooked to the cable by halyard swivels; the surface cage floated in a wooden frame that cast no shadow on the bottles and was tied to the end of a 3 m. floating, bamboo pole, tied in turn to the buoy; cages between

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* The cells were placed for a minute or two in 2 drops of 0·006 *N* iodine in 50 ml. Miquel Sea Water and then returned to pure Miquel. This treatment was suggested by Dr Allen as a result of experiments on the effect of chlorine and iodine on his cultures. Miss D. M. Mees recommended repetition of treatment in 3 days, instead of at the time of the next routine subculture, to prevent spore formation; this led to further improvement in the growth of treated cultures.

† 210 V. lamp run at 100 V. in a dark cellar.

the surface and 5 m. were slung from the pole to avoid the shadow of the buoy.

A 14·5 kg. weight was attached to the cable just below the lowest cage, so that the part of the cable with the bottles attached to it should hang as nearly vertical as possible. As the cable was anchored, strong tides and wind would displace it to a greater or lesser degree from the vertical. No correction has been applied for the unknown extent of this deviation.
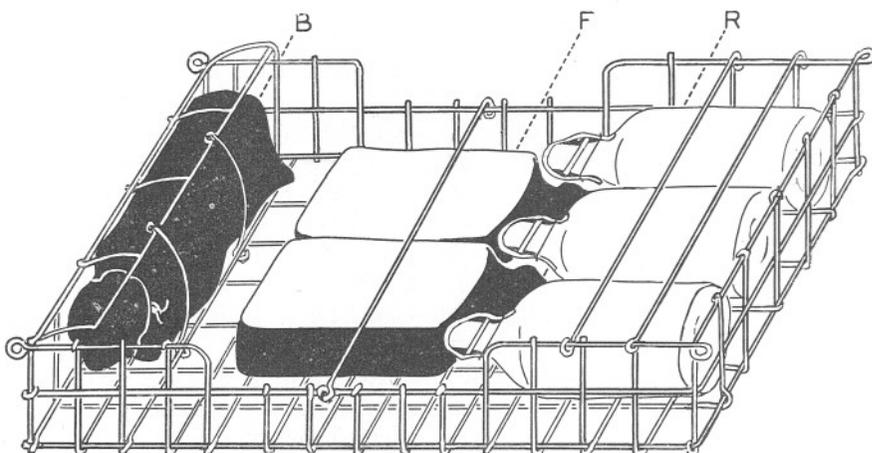
Fig. 2. Cage and bottles in which the diatoms were exposed in the sea (isometric projection). *R*, the three round, clear-glass bottles, exposed to full light; their stoppers are secured by strips of rubber tied over them. *F*, the two flat medicine bottles, blackened on all sides save the uppermost horizontal surface; the contents of these bottles received the same illumination as a photo-electric cell, and were partially shaded, as compared with *R*. *B*, the bottle protected from all light by a black bag of double cloth; this served to measure respiration only.

Lowering or raising the cages took about 2 min., during which time the bottles belonging to the lower levels in the experiment were exposed to considerably greater illumination than at their intended positions. In the 24 hr. experiments in 1934 this error was eliminated by lowering and raising the cages after dusk.

The buoy was anchored at one of two stations in the clear, open, water of the English Channel (Table III), where the transparency of the water did not vary appreciably with the change of the tide, as it might have done nearer the shore. This choice of station made it necessary to choose relatively calm days for the experiments: on rougher days the wind and Atlantic swell coming up the Channel would have made it impossible to shoot or lift the gear safely because of the rolling of the ship. Even the drift due to neap tides, if increased at all by wind, made it extremely difficult to lift. The calmer days in 1933 were also days of almost unbroken sunshine, giving steady illumination that could be accurately measured; in 1934 the calmer days were usually overcast.

TABLE IV. PHOTOSYNTHESIS EXPERIMENT, JULY 25 1933. 0945–1605 G.M.T.
EXPOSURE OF DIATOMS IN "ROUND" BOTTLES ONLY

Initial oxygen content = 4·872, 4·829, 4·801 ml. per litre.
Culture of *Coscinodiscus excentricus* containing 296 cells per ml.

Depth in metres	Oxygen in ml. per litre			Oxygen in ml. per 10^6 cells	Depth in metres	Oxygen in ml. per litre			Oxygen in ml. per 10^6 cells
	Light (l)	Dark (d)	Increase ($l-d$)	Total ($l-d$)		Light (l)	Dark (d)	Increase ($l-d$)	Total ($l-d$)
0	5·547	4·829	0·703	2·38	10	6·348	5·026	1·293	4·37
	5·530		0·686	2·32		6·376		1·321	4·46
	5·482		0·638	2·16		6·370		1·315	4·44
1	5·569	4·812	0·725	2·45	15	5·981	5·048	0·926	3·13
	5·514		0·670	2·26		5·926		0·871	2·94
	5·564		0·720	2·43		5·997		0·942	3·18
2	5·849	4·829	1·005	3·40	20	5·531	5·043	0·476	1·61
	5·706		0·862	2·91		5·613		0·558	1·89
	5·760		0·916	3·10		5·547		0·492	1·66
4	6·271	4·845	1·427	4·82	30	5·130	5·081	0·075	0·25
	6·239		1·395	4·71		5·213		0·158	0·53
	6·244		1·400	4·73		5·141		0·086	0·29
6	6·123	4·878	1·279	4·32	40	5·054	5·075	0·000	0·00
	6·238		1·394	4·71		5·092		0·037	0·13
	6·310		1·466	4·95		5·059	Av. 5·055	0·004	0·14
8	6·178	4·873	1·334	4·51					
	6·190		1·346	4·55					
	6·200	Av. 4·844	1·356	4·58					

Estimation of Oxygen

At the beginning of the exposure in the sea Winkler reagents* were added to at least three "initial" samples of culture; at the end, the reagents were added to all the bottles that had been in the sea, the six in each cage being treated in quick succession with as little further exposure to light as possible. Each bottle was then carefully shaken and sealed with water held in a rubber sleeve surrounding the stopper.

Weather permitting, these reagents were added on board ship, but in any case with the least possible delay, the bottles being kept in the dark in the interval; it was then assumed that any additional oxygen consumption, which might have occurred in that interval, would be the same in the bottles in dark bags and in those which had just been removed from light in the sea, and would therefore cancel out in calculating the total oxygen production in the light. It was found, however, that the respiration of the cultures was barely measurable even after 2 hr. in the dark.

After allowing the precipitate to settle for 1 hr. in the Laboratory, hydrochloric acid was added to the samples, which were then stored under water until required for titration.

In 1934 only 100 ml. from each bottle was titrated in a slow stream of nitrogen; these samples were taken in a random order, to avoid prejudicing the results by knowing the bottle's position in the experiment. In 1933 the bottle's position was known, but the volume of the sample was varied by titrating the entire contents of each bottle.

* 45 % $MnSO_4$, H_2O (40 % $MnCl_2$, $4H_2O$ on July 25 1933); 15 g. NaOH and 5 g. KI in 100 ml. H_2O; $N/100$ KIO_3 as standard; $N/200$ $Na_2S_2O_3$, $5H_2O$ for titration; and 1 % starch solution preserved in 20 % NaCl as indicator.

Calculating and Recording the Results

The oxygen in each bottle was first calculated in ml. per litre at N.T.P., as recorded in Tables IV–VII (pp. 315, 320, 325, 329). These figures were then converted into ml. of oxygen per million diatom cells, so that the results of experiments with different concentrations of diatoms might be compared directly (Table IV).

Then, in order to bring the results into line with those of Marshall & Orr (1928, p. 324) and others, the "total oxygen production" was calculated and recorded in the graphs (Figs. 3–8). "Total oxygen" is obtained by adding to the "net" amount of oxygen produced in the light an amount of oxygen equal to the average amount consumed by respiration in the dark. This calculation is based on the commonly accepted view that plants respire continuously at a constant rate, whether they be kept in the dark or exposed to light. In theory, at least, this assumption may well be queried (Montfort & Neydel, 1928, p. 810); but it is not easy to test in practice, owing to the apparent impossibility of separating the gaseous exchange due to photosynthesis from that due to respiration. As the future may elucidate this problem and show the present assumption to be invalid, the original observations, rather than the derived values plotted in the figures, are recorded for reference in Tables IV–VII.

The amount of oxygen added for respiration, in the present case, is small, and the procedure has the advantage of eliminating negative values for samples exposed to such weak light that oxygen production does not exceed consumption.

There were some minor differences in the treatment of data from the first and last experiments, compared with the rest, to allow for different experimental conditions. For July 25 1933, the total oxygen was taken directly as the difference $(L-D)$ between the oxygen in bottles after exposure to light (L) and dark (D) as in Table IV.

For experiments from August 4 1933 to July 19 1934 a more exact method was used, allowing for differences in the initial values (In) of different portions of the large quantities of culture. The net oxygen production $(L-In)$ was calculated for each bottle in ml. per million cells; the respiration value $(In-D)$ was calculated for the sample at each depth, and then averaged for groups of bottles exposed to roughly similar temperatures (e.g. 0–10 and 12·5–40 m., Tables VI–VII). The total oxygen production, $(L-In)+(In-D)$, was then plotted in Figs. 3–8 against the depth; each bottle exposed to the light being recorded separately to show the degree of divergence due to experimental error.

For the short exposures the results are all plotted on the same scale (Figs. 3–5); for the 24 hr. exposures the oxygen is plotted for convenience half-scale, relative to the depth (Fig. 8).

For August 9–10 1934, when the daytime exposures only lasted for 4 hr. each, the net oxygen production has been plotted (Fig. 6), as the respiration was not measured. Measurements of respiration on the same culture during

the night, showed that it amounted to about 0·09 ml. per million cells in 4 hr., and would therefore have been almost within the general experimental error.

The sea temperatures during the exposures are plotted on the appropriate graphs.

Accuracy of Methods used in Estimating Photosynthesis

The standardization of thiosulphate for titration was accurate on the average to ±0·14%, or about 0·008 ml. per l. O_2. All calculation of oxygen values was therefore carried to three places of decimals (Table IV), and the results recorded after correction to two places. Complete Winkler estimations, carried out upon sets of identical samples in the laboratory, were then found to agree to within ±0·2%, or 0·01 ml. per l. O_2. Similar estimations on triplicate samples of diatom culture, after supposedly identical exposure to light in the sea, agreed, on the average, to within about ±1% in 1933 (0·06 ml. per l. with maximum difference in one case of 0·4 ml. per l.), and less in 1934 (0·05 ml. per l., with maximum in one case of 0·19 ml. per l.). The error here includes discrepancies due to mixing (improved in 1934) and sampling of the diatom culture, any lack of uniformity in behaviour of the diatoms themselves, or in their exposure to light, as well as the inevitable loss of accuracy in the Winkler estimation due to the motion of the ship while the reagents were being added to the bottles.

Respiration values at similar temperatures differed by 0·11 ml. per l. in 1933, and 0·04 ml. per l. in 1934, in any one experiment; for this reason the values have been averaged. Respiration for different cultures varied from 0·02 to 0·12 ml. oxygen consumed per million cells per hour. This large variation may have been due in part to difference in size of the cells, but must also indicate a difference in activity of different cultures, and possibly (as suggested to me by Dr S. A. Waksman) some difference in bacterial infection.

The error in counting the diatoms was well within 1%; the error in sampling was nearly 5%, if 2 ml. samples containing about 600 cells were taken. The absolute error in such a sampling method is of the order of $\sqrt{n}$, where n is the number of the cells in the sample; the error in the present case was, therefore, at least halved by increasing the size of the sample to 5 ml.

The size of the diatom cells varied appreciably in different strains, and that of the same strain decreased as the summer advanced. No allowance has been made for these differences, except in the analyses recorded in the appendix (p. 343) and referred to in the discussion (p. 338). No estimates of dry weight of the diatoms were made.

Factors Influencing the Rate of Photosynthesis During the Exposures at Sea

Although no photosynthesis can occur in the absence of energy in the form of light, it is well known (e.g. Spoehr, 1926, p. 95) that the rate at which it proceeds, even in the presence of light, may be profoundly influenced by a number of other factors, any one of which may act as the "limiting factor" (Blackman, 1905, p. 281) by being present in either an inadequate or excessive amount.

It is, therefore, clearly essential to examine the possible influence of such factors in the present experiments, before considering the effect of energy upon the rate of photosynthesis.

The external factors to be examined are:

(1) The supply of nutrient salts.

(2) The osmotic pressure of the medium.

(3) The hydrogen-ion concentration of the medium (pH).

(4) The partial pressure of the carbon dioxide (CO_2) in the medium.

(5) The temperature.

The internal factors are:

(6) The amount and composition of the chlorophyll, and other pigments in the cells.

(7) Certain protoplasmic, or enzymatic factors.

(8) The accumulation of end-products of photosynthesis either within the cells or in the medium.

Limitations due to anatomical structure and to the translocation of end-products do not arise in the present case, since the plants used were unicellular.

External factors

The Miquel Sea Water medium, in which the diatoms were grown, has been shown to supply them with ample nutrient salts for long-continued growth, as well as a suitable osmotic pressure and pH. All the experiments were carried out in this medium.

The medium apparently supplies an excess of CO_2, for the additional sodium bicarbonate raises the excess base to $2 \cdot 7/1000 N$, the total CO_2* to about 56 ml. per l., and the free CO_2 to about 0·75 ml. per l. when the medium is freshly made up at about pH 7·8. After aeration some CO_2 may be lost, raising the pH to 8·1 or 8·2: but, even then, laboratory experiments showed that this medium could yield about 2·75 ml. per l. CO_2† during a few hours, while in the experiments at sea the amount consumed was never more than 2·0 ml. per l. CO_2, with a rise of less than 0·18 pH. It is still possible that, in

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* I am indebted to Dr L. H. N. Cooper for the data on CO_2, obtained by extrapolation from figures of Buch, Harvey, Wattenberg & Gripenberg (1932, p. 58).

† Assuming a 1 : 1 ratio between CO_2 consumed and O_2 produced.

accordance with Harder's findings (1921, pp. 550 *et seq.*), CO_2 may have been affecting the rate of photosynthesis, for he showed that, even when neither CO_2 nor light was acting as a direct limiting factor, a richer supply of CO_2 would yet lead to higher oxygen production for a given light intensity. This is theoretically important in the present case, but it may be set aside in practice, since the supply of CO_2 in the experimental medium was already richer than would ever be available in the sea naturally; and, even if the values obtained do not show the absolute maximum for oxygen production in the given light intensities, the experiments are still directly comparable with one another.

The temperature of the diatoms during an experiment must have been the same as that of the sea in which they were suspended. They were, therefore, not all at the same temperature, for the English Channel in summer has a thermocline. The greatest difference in temperature, however, between one depth and another in any one experiment was $4.2°$ C. (see Figs. 4, 5). No correction has been made for temperature differences, as no certain data were available as to their effect. If other factors, including light, were favourable, and photosynthesis were being limited by the effect of temperature on the "dark (chemical) reaction", an increase in temperature of $4°$ between 15 m. and the surface might be expected to increase the rate of photosynthesis by about 40%. Figs. 3–7 show that the rate actually decreased towards the surface, and could not therefore have been directly controlled by temperature. On the other hand, the surface temperature may have been so high as to have been damaging the cells, but this appears improbable, since the maximum temperature in the sea was $17.2°$ C. and the cultures were grown successfully in the aquarium at temperatures up to $19°$ C. The harmlessness of the sea temperatures may also be deduced from the successive 4 hr. exposures *A–C* on August 9 1934, when there was no appreciable fall in the rate of photosynthesis towards the surface in the first and last periods, whereas there was a sharp fall in the intermediate midday period. There can hardly have been a significant difference in the temperature of the sea between midday and afternoon, to account for the difference in behaviour of the diatoms.

Internal factors

The only practicable method of controlling the chlorophyll content and other internal factors was always to use actively growing cultures of similar age and closely allied strain and to expose them to identical treatment prior to the experimental exposure.* This at least gave consistent results, whereas cultures of different ages, when compared under controlled light and temperature conditions in the Laboratory, did not give the same ratio between oxygen production per hour and chlorophyll content (as measured by Harvey's method, 1934, p. 770).

* Montfort & Neydel (1928, p. 824) have shown clearly the effect of the prior illumination on the rate of photosynthesis.

The accumulation of end-products of photosynthesis within the cell should lead to cell division and growth, and there is no reason to suppose that this normal process was interfered with in the experiments: but no data are known that would show how closely cell division keeps pace with photosynthesis in these diatoms, or how far their rate of photosynthesis may be retarded by increase of end-products towards the end of a long exposure. The diatoms were kept in the dark for some hours before each experimental exposure so as to eliminate previously accumulated products as far as possible. Therefore their

TABLE V. PHOTOSYNTHESIS EXPERIMENTS, AUGUST 9–10 1934

Depth in metres	Period				Depth in metres	Period			
	A	B	C	D		A	B	C	D
	Oxygen in ml. per litre, after exposure to light					Oxygen in ml. per litre after exposure in dark			
0	6·56	5·66	6·05	5·31	0	—	—	—	5·14
	6·58	5·72	6·03	5·31	25	—	—	—	5·17
	6·51	5·71	6·04	5·31	35	—	—	—	5·18
2·5	6·22	5·85	5·97	Lost					
	6·31	5·83	5·99			Oxygen in ml. per litre in initial samples			
	6·30	5·89	5·93						
5·0	6·57	6·25	5·95	Lost		4·98	5·13	5·17	5·22
	6·62	6·12	5·99			4·98	5·18	5·09	5·24
	6·58	6·18	6·00			4·96	5·12	5·20	5·25
10	6·10	6·09	5·74	Lost		—	5·02	—	5·31
	6·26	6·06	5·77						
	6·20	6·04	5·73			Number of diatom cells per ml.			
17·5	5·63	5·66	5·39	Lost					
	5·60	5·55	5·40			672	386	411	331
	5·61	5·59	5·40						
25	5·30	5·25	5·25	5·16		Sunrise at Plymouth 0456 G.M.T.			
	5·27	5·32	5·25	5·17		Sunset at Plymouth 1946 G.M.T.			
	5·24	5·33	5·21	5·17					
35	—	5·20	—	5·18					
	—	5·22	—	5·17					
	—	5·21	—	5·19					

re-accumulation only seems likely to have had an appreciable effect upon the rate of photosynthesis towards the end of the whole-day exposures.

The accumulation of oxygen, as an end-product, in the surrounding medium may have a retarding effect upon the rate of photosynthesis, for Spoehr (1926, p. 169) quotes results to show that the rate in air may be reduced to one-third by an increase in the partial pressure of oxygen from 0·02 to 1 atmosphere. Retardation would be very slight in the present experiments, since the greatest increase in the partial pressure of oxygen in the medium, during an exposure, was less than 0·1 atmosphere.

It therefore appears that the factors which may possibly have been limiting the rate of photosynthesis in the present case were shortage of CO_2, low temperatures in the deeper exposures, and accumulation of end-products.

Now the characteristic "limiting factor" of Blackman (1905, p. 289) would check the rate of photosynthesis at a steady maximum, despite the further increase in amount of other favourable factors. It is clear that in the results shown in Figs. 3–7 no such limiting factor is directly in control of the oxygen production, for after reaching a maximum there comes a point when further approach towards the surface, with increasing light intensity, does not even maintain a steady rate of oxygen production but results in a marked decrease.

Fig. 3. Photosynthesis experiment with *Coscinodiscus excentricus* at L 5 on July 25 1933, using round bottles only. Each spot shows the total oxygen production (*l–d*) as measured in one of the three round bottles at any depth. The plain curve shows the total available energy (energy integral) during the exposure, as computed from measurements of the illumination integral and the extinction coefficients for blue, green, yellow, and red light. The exposure lasted from 0945 to 1605 G.M.T.
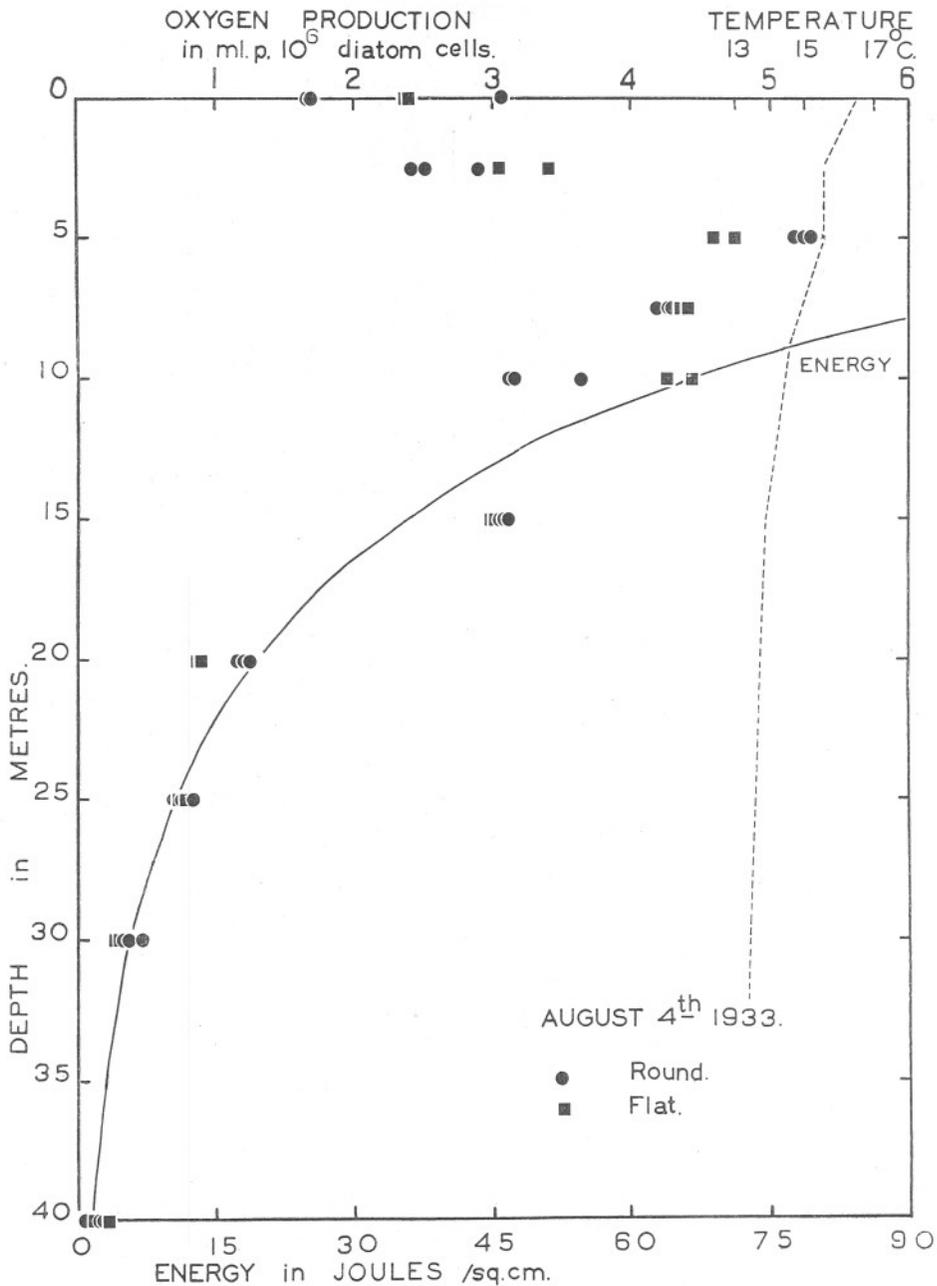
Fig. 4. Photosynthesis experiment with *Coscinodiscus* at L 5 on August 4 1933, shown on exactly the same scale as Fig. 3. The exposure lasted from 0910 to 1445 G.M.T. The round spots represent the total oxygen production (*l–In*) plus (*In–d*), in round bottles, and the squares that in flat bottles. The plain curve represents the total available energy, computed as for Fig. 3. The dotted curve represents the temperature of the sea during the experiment.

The measurements of both the photosynthesis and the energy in the sea may therefore be examined on the assumptions that the illumination is the only remaining variable upon which the rate of photosynthesis can be dependent, and that changes in the wave-length of the illumination do not affect the rate so long as the total energy in the illumination remains the same.

RESULTS OF THE EXPERIMENTS

On the first five occasions recorded here, the position chosen for the experiment was close to Station L 5 by the Eddystone Lighthouse; on the sixth and last occasion, August 9–10 1934, the position lay to the south of Stoke Point, 4 miles south by east of the Shagstone.

The experimental exposures may best be considered in four groups, according to the time of day at which they were made and their duration; there were three 5–7 hr. exposures round midday in July and August 1933; three successive 4 hr. exposures during daylight on August 9 1934; one night exposure of 11·5 hr. on August 9–10 1934; and two 24 hr. exposures in July 1934.

Three short Exposures in 1933 (Tables III, IV and VI, and Figs. 3–5)

The exposures were made on July 25 (Fig. 3), August 4 (Fig. 4) and August 28 (Fig. 5), and lasted between 5 and 7 hr. round midday. The weather was comparatively fine on each day (Table III), but was especially calm on July 25.

The plain curves in Figs. 3–5 represent the total energy integrals (p. 307) in joules, available during the exposure, plotted against the depth in metres. The amount of energy decreases almost exponentially with depth (Fig. 1).

The circles in Figs. 3–5 show the total oxygen production per million diatom cells in round bottles. Below a certain depth it can be seen that the oxygen values fall fairly closely on the curves for energy; this critical depth varies from 10 to 15 m. on the different days, but corresponds to an energy integral of about 7·5 joules or 1·8 g.-cal. per cm.^{2} per hr. on each occasion. Above this depth the oxygen values fall short of the energy curve, the divergence being greatest towards the surface, thereby suggesting an inhibitory effect due to the stronger light.

On August 4 and 28 flat, partially shaded, bottles (Fig. 2, *F*) were exposed, as well as the round bottles; the results are shown by the black squares, and are interesting in connexion with this possible inhibitory effect. At the lower levels the flat bottles give results that are not significantly different from those in the round bottles; but, in the upper layers where inhibition is suggested, there is a distinct tendency for the shaded bottles to give a higher value for oxygen production than the fully illuminated round bottles (Fig. 5), thereby supporting the hypothesis which attributes the inhibition to too much light, rather than to any effect of temperature.
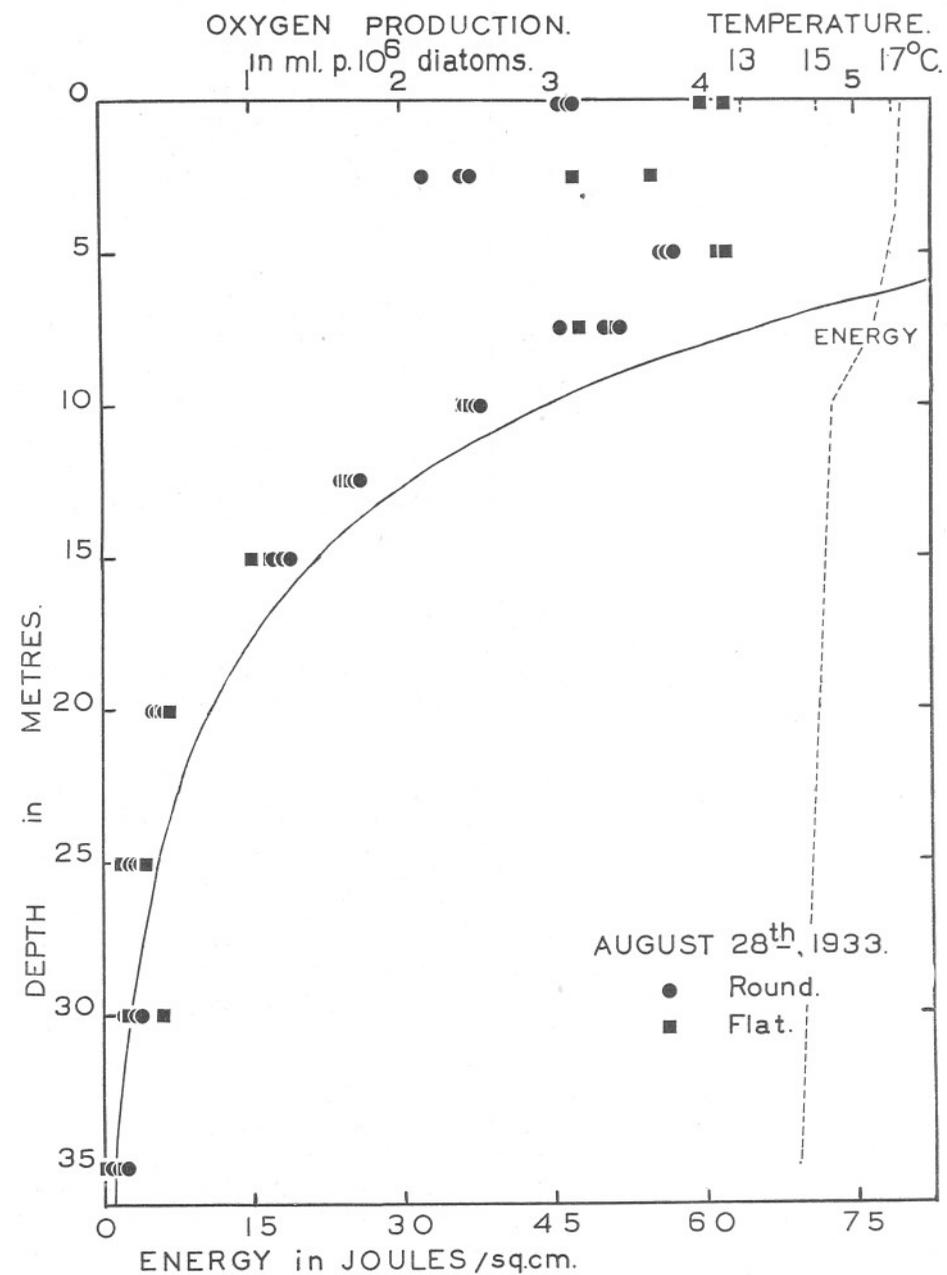
Fig. 5. Photosynthesis experiment with *Coscinodiscus* at L 5 on August 28 1933, shown on the same relative scales as Figs. 3 and 4. The exposure lasted from 1140 to 1650 G.M.T. Symbols and curves as in Fig. 4.

Successive four-hour Exposures in 1934 (Tables III and V and Figs. 6 and 7)

Three successive 4 hr. exposures were made on August 9 1934, starting at 0630 G.M.T. (Table III). The sky became increasingly overcast and the sea grew rougher as the day passed.

The plain curves in Fig. 6 again represent the energy integrals as computed from the photo-electric measurements made during the exposures.

TABLE VI. PHOTOSYNTHESIS EXPERIMENTS, AUGUST 1933. EXPOSURE OF DIATOMS IN ROUND BOTTLES (*R*) AND FLAT BOTTLES (*F*) (SEE FIG. 2)

Depth in metres	Bottle	Oxygen in ml. per litre Aug. 4 0910–1445 G.M.T. Light (l)	Dark (d)	Aug. 28 1140–1650 G.M.T. Light (l)	Dark (d)
0	R	5·35 / 5·34 / 5·75	4·87	6·36 / 6·34 / 6·39	5·12
	F	5·56 / 5·57	—	6·48 / 6·42	—
2·5	R	5·57 / 5·60 / 5·70	4·80	6·10 / 5·99 / 6·01	5·14
	F	5·86 / 5·76	—	6·13 / 6·31	—
5	R	6·41 / 6·37 / 6·41	4·82	6·62 / 6·64 / 6·65	5·18
	F	6·21 / 6·25	—	6·47 / 6·48	—
7·5	R	6·09 / 6·12 / 6·12	4·84	6·47 / 6·51 / 6·35	5·13
	F	6·16 / 6·15	—	6·24 / 6·15	—
10	R	5·92 / 5·78 Av. / 5·78	4·93 / 4·85	6·09 / 6·12 / 6·12	5·13
	F	6·17 / 6·11	—	5·91 / 5·89	—
12·5	R	—	—	5·81 / 5·81 / 5·76	5·03
	F	—	—	5·62 / 5·61	—
15	R	5·80 / 5·81 / 5·81	4·90	5·56 / 5·62 / 5·61	5·11
	F	5·76 / 5·76	—	5·44 / 5·39	—
20	R	5·26 / 5·26 / 5·24	4·92	5·27 / 5·24 Av. / 5·24	5·03 / 5·11
	F	5·14 / 5·14	—	5·20 / 5·19	—

Depth in metres	Bottle	Oxygen in ml. per litre Aug. 4 0910–1445 G.M.T. Light (l)	Dark (d)	Aug. 28 1140–1650 G.M.T. Light (l)	Dark (d)
25	R	5·14 / 5·11 / —	4·90	5·12 / 5·12 / 5·12	5·02
	F	5·08 / 5·09	—	5·15 / 5·09	—
30	R	5·00 / 5·04 / 5·00	4·89	5·13 / 5·12 / 5·10	5·06
	F	4·96 / 4·96	—	5·07 / 5·06	—
35	R	—	—	5·07 / 5·08 Av. / 5·06	5·05 / 5·04
	F	—	—	5·05 / 5·02	—
40	R	4·95 / 4·95 / 4·92	4·89	—	—
	F	4·95 / 4·92	—	—	—
50	R	4·90 / 4·94 Av. / 4·92	4·91 / 4·90	—	—
	F	4·92 / 4·90	—	—	—

Initial contents	Bottle	O₂ (In)	Cells per ml.	O₂ (In)	Cells per ml.
0–20	R	4·87 / 4·90	293	5·25 / 5·26	405
25–50	R	4·90 / 4·93	293	5·18 / 5·19	405
0–50	F	4·87 / 4·92	293	5·20 / 5·15	345

Joules are plotted on the same scale, relative to depth, as in the previous graphs; but the energy integrals are slightly magnified by making no deduction for reflexion loss; a second curve ($C \times 0.66$, Fig. 6) for the third period shows the energy integrals after deducting 33 % for reflexion loss. This deduction is partly based upon photographic measurements of the subsurface illumination integral (p. 310).

The net (not "total", see p. 316) oxygen production is shown by squares for the morning period *A*, by circles for the midday period *B*, and triangles for the afternoon period *C*. For simplicity only the results obtained in round

bottles are shown; but they were confirmed by flat bottles, as in the August experiments of the previous year. Below about 15 m. (Fig. 6) the oxygen values again fall fairly close to the energy curve for the corresponding period. Above 15 m. the oxygen values for the early morning and afternoon periods, A and C, increase slightly up to 5 m., though falling far behind the energy curve, and then remain practically constant to the surface, in spite of the rapid increase in energy: the oxygen values for the midday period, B, increase in the same way up to 5 m.; but, above this, they show a marked decrease towards the surface, just as in the experiments in the previous year, when the light at the surface was bright (Figs. 3–5). The coincidence of all values at 2·5 m. would seem to be fortuitous.

The oxygen values for period C fall closer to the curve $C \times 0·66$, than to the curve $C \times 1$. Though a 33 % reflexion loss is rather high, it seems more accurate to assume some such change in the subsurface illumination (p. 310) than to postulate a change in the activity of the diatoms used in the third period, as compared with those used in the other periods, since diatoms of the same age and strain were used for all three periods. All the oxygen values are high during these exposures compared with the previous year (p. 311).

Night Exposure in 1934 (Tables III and V, and Fig. 6)

The night exposure (period D) followed immediately on the 4 hr. daytime exposures; it started at 1835 G.M.T. on August 9 and lasted 11·5 hr., that is until 0608 on August 10. The sea conditions were considerably rougher than during the preceding day and three of the cages of bottles were lost, so that no results were obtained from depths between the surface and 25 m. At 25 and 35 m. the oxygen content of the clear glass bottles was the same as that of the darkened bottles, showing that although the exposure started 2 hr. before sunset and lasted until 2 hr. after sunrise, the amount of energy penetrating to a depth of 25 m. or below, was insufficient to produce a detectable quantity of oxygen by photosynthesis. There was a slight production of oxygen at the surface during this period, agreeing fairly closely with the measure of available energy.

The results for these successive 4 hr. periods, and the night period, covering together 23·5 hr., are replotted in Fig. 7, with the oxygen as ordinates, and the time of day as abscissae, for the various depths. The relation of the oxygen production to the waxing and waning of daylight is clearly shown, as well as the midday inhibition near the surface; this result is closely similar to that obtained by Marshall & Orr (1928, fig. 7, p. 328) for a sunny summer day in the Clyde. The similarity is emphasized by the similar method of plotting, which allows the two sets of results to be compared directly.
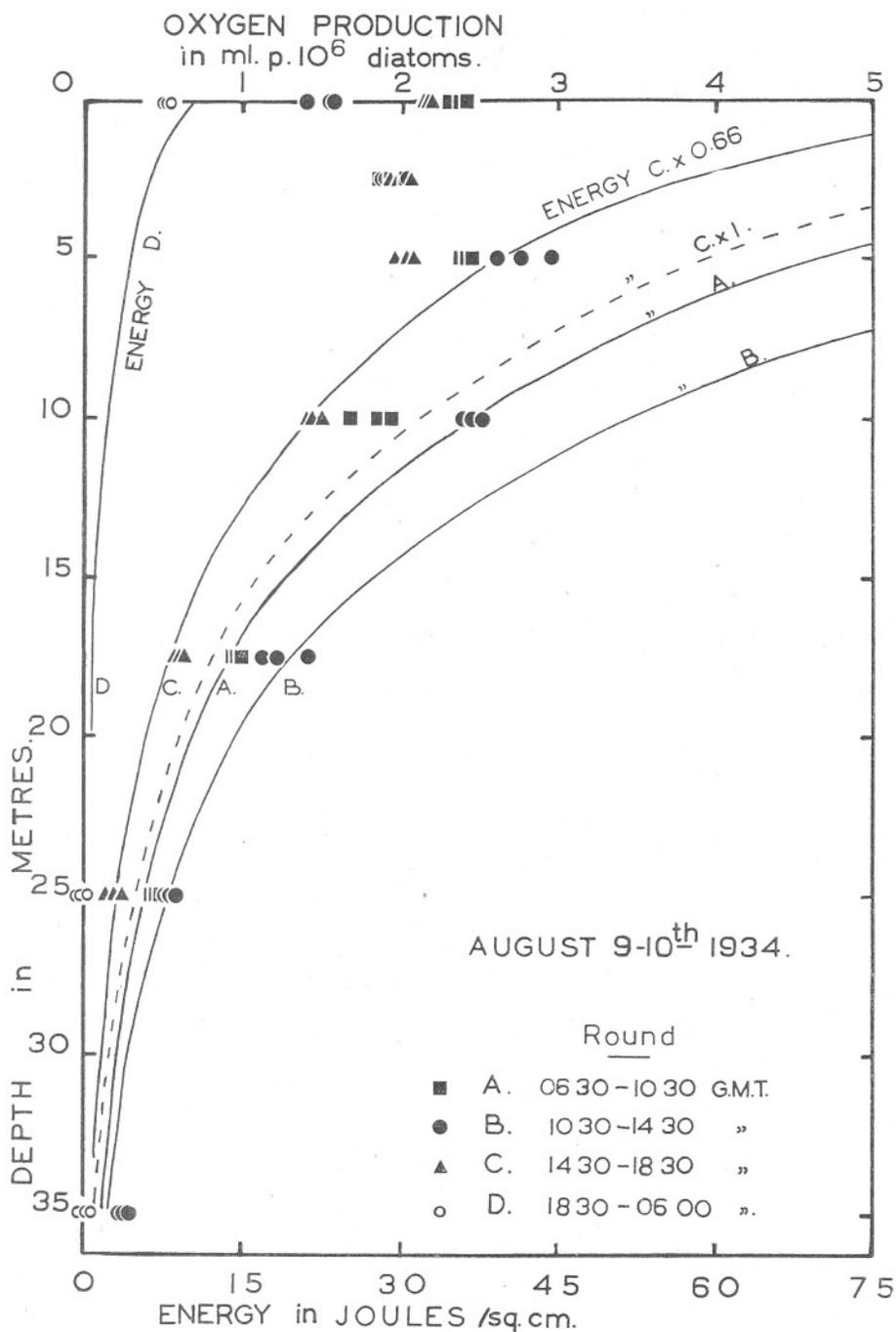
Fig. 6. Photosynthesis experiments with *Coscinodiscus* off Stoke Point on August 9–10 1934, shown on the same relative scales as Figs. 3–5. The exposures were made in three successive 4 hr. periods, *A–C*, during the day, and an 11·5 hr. period covering the night; period *A* lasted from 0630 to 1028 G.M.T., period *B* from 1032 to 1430, period *C* from 1434 to 1828, and period *D* from 1835 to 0608. The net oxygen production (*l–In*) is shown, for round bottles only, by squares for period *A*, rounds for *B*, triangles for *C*, and rings for *D*. The total available energy is shown without subtraction of surface loss (p. 310) for periods *A*, *B* and *C* (curves *A*, *B* and *C* × 1), and after subtracting 33 % surface loss for period *C* (curve *C* × 0·66). The temperature of the sea, on the morning of August 9, was 14·75° C. at 5 m., 14·40° C. at 15 m., 13·78° C. at 25 m., and 13·57° C. at 45 m.

Twenty-four-hour Exposures in 1934 (Tables III and VII and Fig. 8)

The first of these exposures began at 2120 G.M.T. on July 12 and lasted 23 hr. 40 min.; the second began at 2035 on July 19 and lasted 22 hr. 40 min. Both were slightly curtailed by the onset of rough weather (Table III).
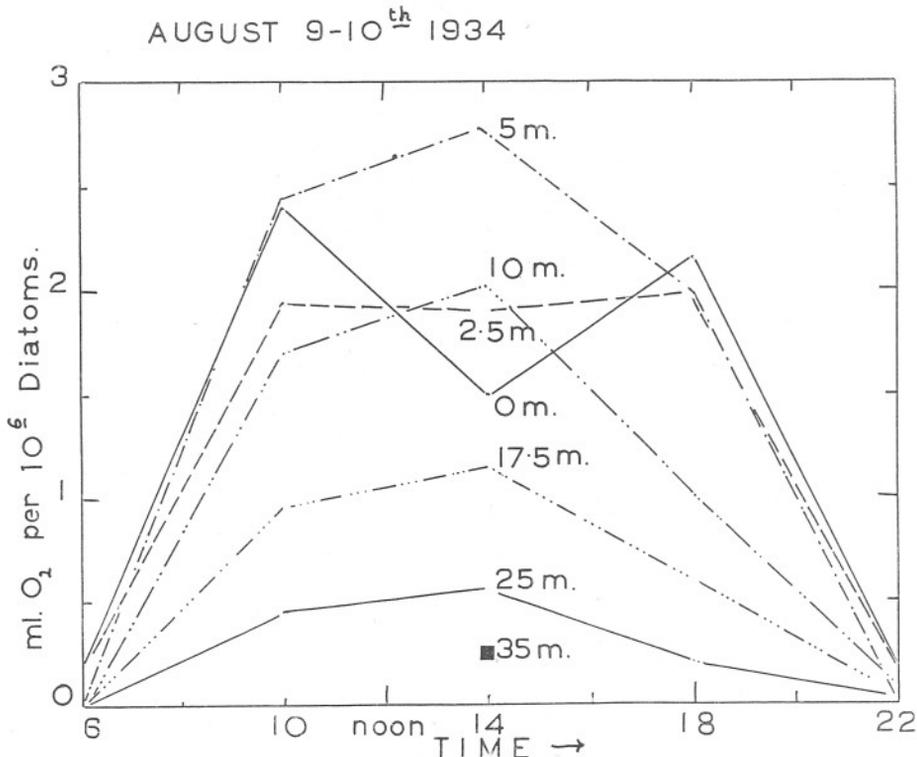
Fig. 7. Photosynthesis experiment with *Coscinodiscus* off Stoke Point on August 9–10 1934. The results of this experiment, shown in Fig. 6, are here plotted with the oxygen-production in ml. per million diatom cells as ordinates and the time of day as abscissae. The curves represent the results at each depth, as indicated. This method of showing the results is directly comparable with Marshall & Orr's figs. 7 and 10 (1928, p. 328).

The plain curve in Fig. 8 represents the energy integrals for July 12–13, plotted half-scale as compared with previous figures. The energy for the experiment on July 19–20 is shown by small circles, as the values are almost identical with those shown by the curve (see p. 309 and Table III).

The total oxygen production during the first experiment is shown by solid circles and squares, that for the second by rings and crosses. The oxygen values, like the energy values, are plotted half-scale as compared with previous figures; the relative scale of oxygen and energy in Fig. 8 is therefore unaltered.

The figure shows that a similar relation between oxygen production and energy holds good for these long exposures, as for the short exposures; but there are greater discrepancies. This is partly to be expected on account of the increased opportunity for accumulation of end-products in both diatoms and medium during the longer exposures (p. 320).

TABLE VII. PHOTOSYNTHESIS EXPERIMENTS, JULY 1934. EXPOSURE OF DIATOMS IN ROUND BOTTLES (R) AND FLAT BOTTLES (F)

Depth in metres	Bottle	Oxygen in ml. per litre July 12 and 13 2120–2100 G.M.T. Light (*l*)	Dark (*d*)	July 19 and 20 2035–1915 G.M.T. Light (*l*)	Dark (*d*)
0	R	6·21 6·23 6·21	4·89	Lost	Lost
	F	5·95 5·97	—	—	—
2·75 (12th) 2·5 (19th)	R	5·76 5·48 5·70	4·88	6·02 6·12 6·08	5·04
	F	5·83 5·79	—	5·64 5·66	—
5·0	R	6·92 6·92 6·93	4·88	6·73 6·74 6·71	5·02
	F	6·23 6·28	—	5·73 5·76	—
7·5	R	6·89 6·70 6·90	4·89	6·65 6·66 6·65	5·08
	F	6·26 6·25	—	5·69 5·73	—
10	R	6·52 6·50 Av. 6·52	4·88 4·88	6·57 6·58 Av. 6·55	5·03 5·04
	F	6·15 —	—	5·66 5·69	—
12·5	R	6·36 6·38 6·34	4·89	6·49 6·42 6·45	5·05
	F	6·05 6·06	—	5·59 5·57	—
15	R	6·17 6·25 6·25	4·90	6·25 6·29 6·27	5·07
	F	5·94 5·90	—	5·55 5·56	—
17·5	R	6·39 6·40 6·34	4·88	—	—
	F	5·88 5·85	—	—	—

Depth in metres	Bottle	Oxygen in ml. per litre July 12 and 13 2120–2100 G.M.T. Light (*l*)	Dark (*d*)	July 19 and 20 2035–1915 G.M.T. Light (*l*)	Dark (*d*)
20	R	6·09 6·03 Av. 6·08	4·90 4·89	6·00 5·99 6·01	5·09
	F	5·72 5·72	—	5·46 5·45	—
25	R	5·89 5·82 5·79	4·91	Lost	Lost
	F	5·53 5·53	—	,,	—
30	R	5·67 5·71 Av. 5·62	4·92 4·92	—	—
	F	5·31 5·30	—	—	—
35	—	—	—	Lost	Lost
45	R	—	—	5·12 5·14 Av. 5·13	5·06 5·07
	F	—	—	5·15 5·16	—

Depth in metres	Bottle	Initial contents O₂ (*In*)	Cells per ml.	O₂ (*In*)	Cells per ml.
0–45	R	4·96 (*l*) 4·96 (*l*) 4·99 (*d*) 4·99 (*d*)	150 139	5·12 5·10 5·13 5·11	163
0–45	F	4·94 4·94 4·95	108	5·18 5·16 5·18 5·16	53

Sunrise 0410 G.M.T.   0426 G.M.T.
Sunset 2031 ,,   2016 ,,

Apart from the irregularities in the results, especially for July 12, there remains an obvious discrepancy between the amount of oxygen produced by a million cells in similar illumination in the two experiments, that for July 12 being distinctly the greater. There is evidence that this may have been due to the size of the individual cells: first, although the cells were not measured, those in the culture for July 12 were recorded as unusually large, whereas those for July 19 were not; and, second, the average respiration rate of a million cells per hour was about 0·025 ml. for July 12 and only 0·017 ml. for July 19 at similar depths. If this be taken as indicative of the relative sizes of the cells in the two cultures, the oxygen production for July 12 can be reduced
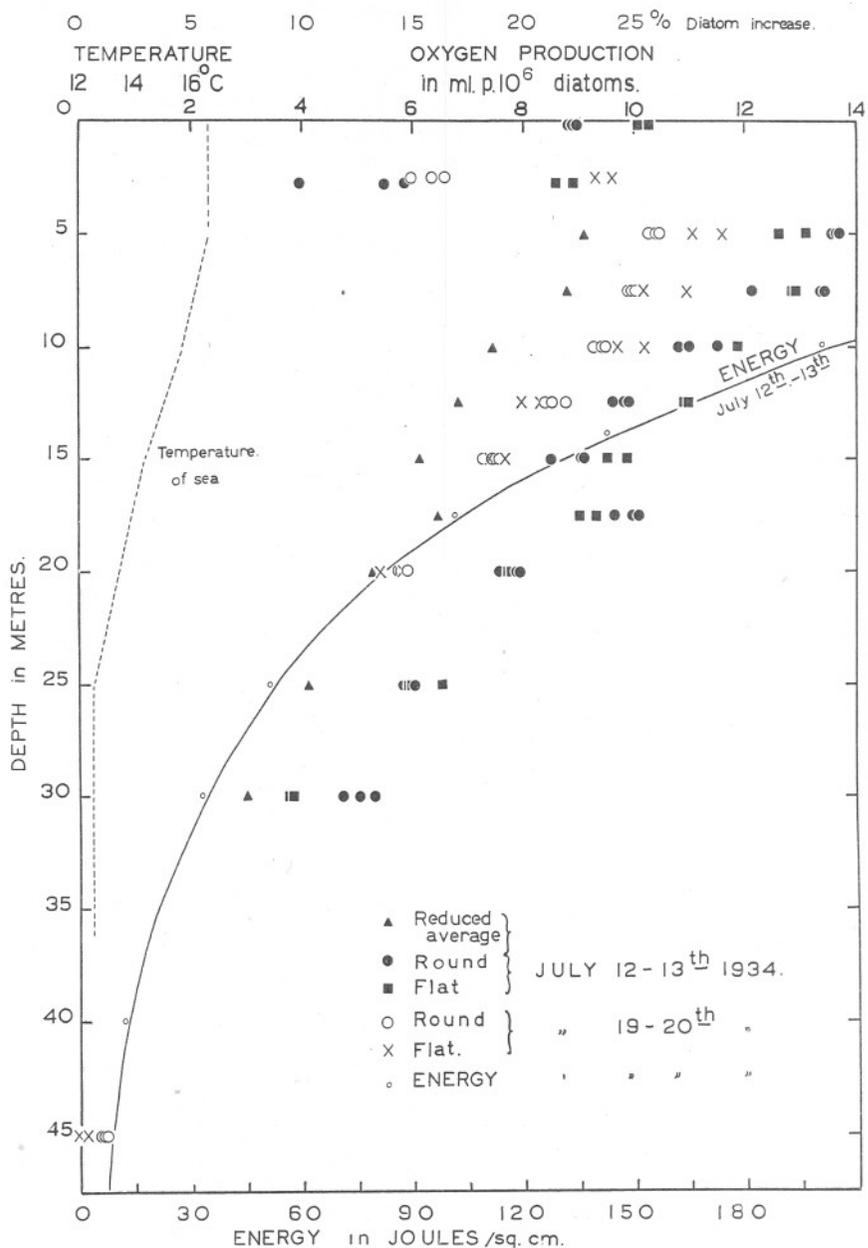
Fig. 8. Photosynthesis experiments with *Coscinodiscus* at L 5 on July 12–13 and July 19–20 1934. The exposures lasted about 24 hr. each, the first from 2120 to 2100 and the second from 2035 to 1915 G.M.T. The scales on which total oxygen production and total available energy are plotted are halved in relation to depth, as compared with Figs. 3–6, but remain the same relative to one another. The rounds and squares represent the results with round and flat bottles for July 12–13, the rings and crosses those for round and flat bottles for July 19–20. The triangles represent average values for July 12 reduced to the same level as for July 19, in accordance with respiration values (see text, p. 331). The plain curve represents the total available energy on July 12–13; the small rings indicate the closely similar values for July 19–20. The dotted curve represents the temperature of the sea on the evening of July 19.

accordingly and then compared with that for July 19. This gives the average values plotted as triangles in Fig. 8, where they show a close approximation to the values for July 19 except that the curve is slightly steeper. The latter point and the other irregularities in the results suggest that strong currents, due to the spring tide, may have been putting a heavy strain on the buoy, at least during a part of the exposure on July 12–13. This would displace the cable considerably from the vertical, and bring the cages nearer to the surface than was intended.

Near the surface the flat and partially shaded bottles again tend to show higher values for oxygen production than the clear round bottles at the same levels (p. 323).

On July 12 no cages were exposed below 30 m., but this proved unexpectedly to be well above the "Compensation Point" (p. 336), where photosynthesis just balances respiration in the 24 hr. On July 19 cages were set down to 45 m., and the bottles there showed that the oxygen production just balanced the consumption (Table VII), so that the 45 m. cage must have been close to the compensation point: unluckily the cages at 25 and 35 m. were lost, owing to bad weather, and their confirmation is therefore lacking. The corrected values for July 12 serve as confirmation as far as they go; and it is found that a similar depth for the compensation point is obtained by combining the results of the four successive exposures covering 23·5 hr. on August 9–10 1934.

THE RELATION OF PHOTOSYNTHESIS TO ENERGY IN THE SEA

It has been assumed, in accordance with the conclusions of Stanbury (1931, p. 651), that the energy in radiation of all wave-lengths, within the visible spectrum, may be used equally effectively by the diatoms for their photo-synthesis. Hoover (1937, p. 6) has since shown that the entire visible spectrum is effective for photosynthesis by wheat seedlings, but that there is a principal maximum efficiency for equal incident energy at 6550 A. in the red, and a secondary one at 4400 A. in the blue; between these wave-lengths a fairly high level of photosynthesis is attained throughout, and outside these limits the level falls rapidly to zero.

In the English Channel radiant energy in the blue and yellow regions of the spectrum penetrates into the sea at almost the same rate as that estimated for the total energy, and the rate for green is not widely different (Fig. 1). It is, therefore, possible that the apparent correspondence, to be seen in Figs. 3–8, between oxygen production by diatoms and the total energy, might only imply a correspondence with energy in the blue, or the yellow, or the green, or any combination of these wave-lengths. At present there seems to be no evidence to decide this point; but one thing is quite certain, in the present case, and that is that the photosynthesis is not solely, nor even mainly, dependent upon energy in the red, because the red is practically extinguished within 12–15 m. from the surface of the sea, whereas the diatoms assimilate

actively at depths of 40 and even 50 m. Atkins & Poole (1933, p. 156) made a similar deduction from Marshall & Orr's results in the Clyde.

Accessory yellow and brown pigments are especially abundant in red and brown algae and in diatoms, and it has frequently been suggested, though never fully substantiated, that the energy in light of complementary colour, absorbed by these pigments, may be used in photosynthesis. On the other hand, Schmucker (1930, p. 851) claimed that the energy absorbed by these pigments is thereby removed from the field of photosynthetic activity, rather than made available for it (Warburg & Negelein, 1923, p. 191). Positive evidence that yellow pigments in etiolated leaves would liberate oxygen in the light, when no chlorophyll was present, has been obtained by Smith (1930, p. 148): he also emphasized, in reviewing the chemical nature of these pigments, the ease with which many of them might apparently be formed from, or transformed into, chlorophyll. Moreover, definite colour changes have been described in diatoms by Stanbury (1931, p. 651) and in species of the blue-green alga *Oscillatoria* by Gaidukow (1903, p. 487); these changes were complementary in colour to that of the light in which the cultures were grown, and were apparently due to a greater or lesser development of brownish pigments, masking the green of the chlorophyll. It is tempting to believe that such changes are an adaptation to aid in photosynthesis, and to suppose that, if the time required for the colour change to occur were a matter of days, diatoms, growing in the sea and adapted to blue-green light by the development of brownish pigment, should be able to produce more oxygen, for a given intensity of daylight, in deep water where blue-green light predominates, than should the experimental diatoms, grown in daylight and adapted to a high proportion of red light by development of mainly the green pigments.

But it is necessary to pass on from such speculations on the wave-length of the light to the consideration of its intensity in relation to photosynthesis.

In plotting the total available energy and the oxygen production of *Coscinodiscus excentricus*, the measured or computed amounts for each exposure were shown in Figs. 3–8, as already described. The general relation between energy and oxygen production has been pointed out: there is a direct relation between the two in deep water; the oxygen reaches a maximum at about 5 m. below the surface, and usually decreases above this level, if the light is at all bright. But in the experiments so recorded the length of time for which the diatoms were exposed differed considerably; and it is clearly necessary to eliminate this time factor in order to obtain a true comparison between the different exposures. This is done in Fig. 9, where the oxygen production per hour is plotted as ordinates against the average amount of energy per hour as abscissae. For simplicity the results from each set of three bottles, similarly exposed, have been averaged.

When the flux of energy is below about 7·5 joules, or 1·8 g.-cal. per cm.[2] per hr., there is the usual straight line relation between the energy and the oxygen production, as measured in the six short exposures in two successive
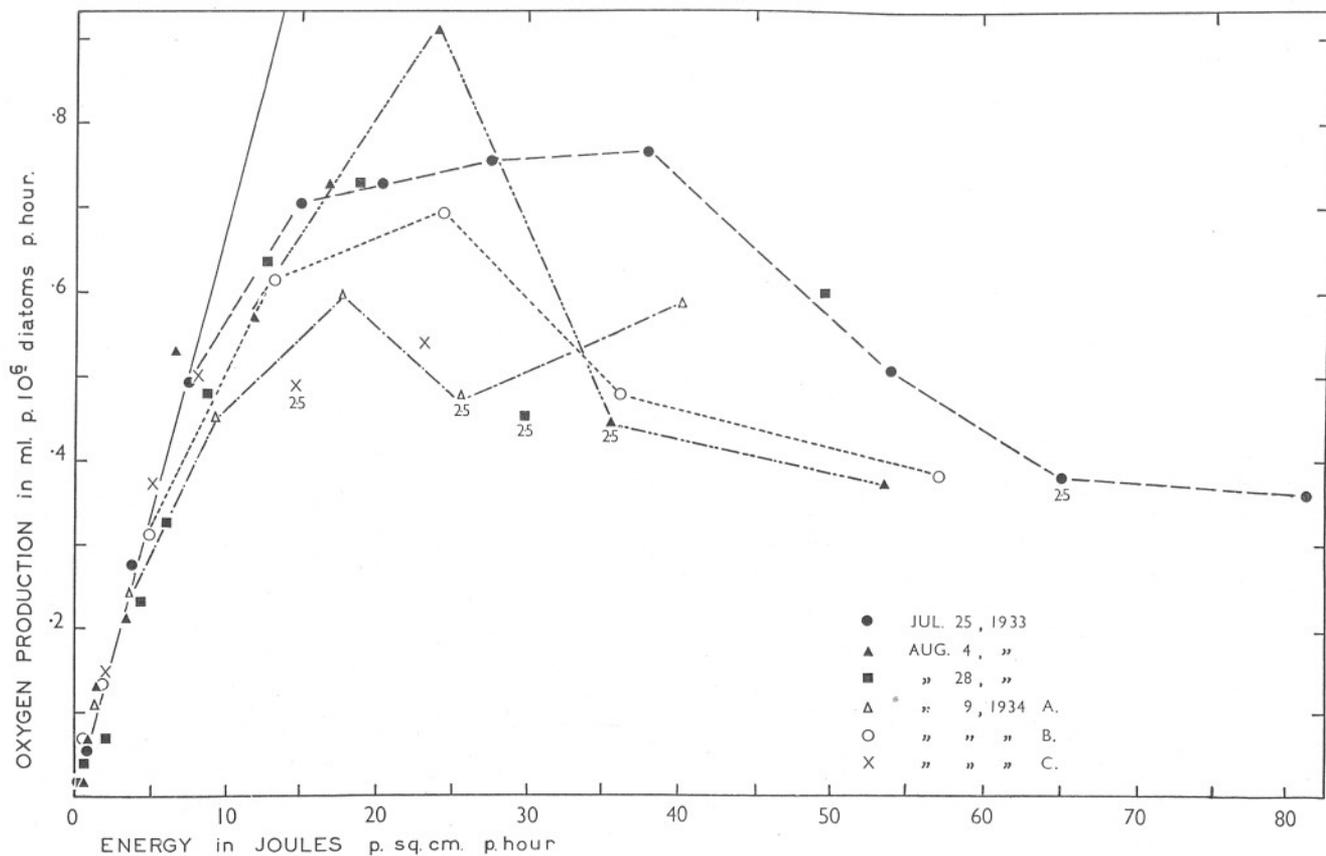
Fig. 9. The hourly oxygen production and total available energy in all short exposures in 1933 and 1934. The straight line from the origin indicates the direct relation between oxygen production and energy at intensities less than about 7·5 joules per hr. At greater intensities the relation in different experiments is indicated by broken lines. The sea was very calm, the light was very bright, and conditions were relatively constant throughout the exposure on July 25; the conditions were probably least constant on August 9. The results obtained in the cages suspended at 2·5 m. are marked 2·5 (see text, p. 335). The energy values for August 9 are derived from curves *A*, *B* and *C* × 0·66 in Fig. 6.

years (Fig. 9). It is hardly to be expected that the agreement should be more exact, considering the difficulties of work at sea, and the important fact that the energy was by no means constant at the value recorded during any one exposure. It is rather a matter for surprise that the agreement is so good, when the energy flux varied so much with the fluctuations in intensity of daylight: but it must be remembered that the energy values for August 9 are slightly magnified, relative to the rest (p. 325).

It is possible to estimate quite roughly from this straight-line relation the actual amount of energy available for absorption for a given oxygen production, and thence to calculate the "percentage utilization" of the energy (Juday & Schomer, 1935, p. 76). Assuming that the cells are so thinly scattered in the experimental bottles that none casts a shadow on any other, and that all are lying horizontally, we may estimate the area of the upper exposed surface of a million cells at about 20 cm.^{2}, if the diameter of each cell is between 50 and 60 μ. At a flux of 7·5 joules the oxygen production of a million cells is about 0·5 ml. per hr.: the amount of energy available over an area of 20 cm.^{2} in 1 hr. would be 150 joules, or 300 joules for 1·0 ml. of O_2. This is equal to 72 g.-cal. If the energy required to form a gram molecule of glucose be taken as 676,000 g.-cal., and is accompanied by the liberation of 22·4 × 6 l. O_2, then the energy required for the production of 1 ml. O_2 would be about 5 g.-cal., and the utilization would amount to about 7 % in those cases falling on the straight line in Fig. 9. Juday & Schomer (1935, p. 80) give the maximum utilization in their exposures as about 11 %.

When the flux of energy is greater than 7·5 joules per hr., the results are less consistent, although most of them show that there is an optimum illumination for oxygen production, somewhere between 20 and 30 joules per hr. (4·8–7·2 g.-cal.), while at greater intensities there is a marked decrease in oxygen, rather than a maintenance of the optimum level as would be expected under the influence of any direct limiting factor, other than light.

The most consistent results were those obtained from the exposure on July 25 1933, when the conditions were as nearly steady as could be expected in the field: the sea was as calm as the traditional mill-pond, and the record of the illumination showed a steady curve throughout the exposure, corresponding to the cloudlessness of the sky. In this case the oxygen production curve (Fig. 9) begins to diverge from the straight line at about 10 joules per hr. (2·4 g.-cal.), rises slowly to an optimum between 20 and 40 joules per hr., falls again till the energy reaches about 60 joules per hr. (14·4 g.-cal.) and then flattens out.

The form of the curves for August 4 1933, and for period B on August 9 1934 (Fig. 9), are both similar to that for July 25, but the first has a higher maximum, and both show the decrease at about 25 joules instead of 40. In both the variations in energy during the exposure were much greater than on July 25, and presumably therefore included moments of much brighter illumination.

The results for the three remaining exposures, August 28 1933, and periods *A* and *C* on August 9 1934, are less regular, having aberrant low values for the bottles suspended at 2·5 m. (the same is also true to a lesser extent for August 4 and for period *B*, August 9). The same discrepancy in the 2·5 m. values can be seen in Figs. 4–8. The cage containing these bottles was suspended below the bamboo, connecting the surface cage to the buoy, and not under the buoy; for it had been found by experiment that bottles suspended at 2·5 m., under the buoy, gave distinctly higher values for oxygen production than those under the bamboo. It was concluded that shading by the buoy reduced the usual inhibitory effect of the strong light near the surface, and that the bottles under the bamboo were therefore giving the truer value. It is difficult to account for these bottles showing greater inhibition than those at the surface; but it is important to notice that these aberrant values occurred on the rougher days. It is perhaps plausible to suggest that passing waves alternately cast shadows and focused bright shafts of light upon these bottles, so that they had intervals of exposure to very much brighter illumination than the average value shown for the energy in Fig. 9.

Inhibition of photosynthesis in bright light (p. 323) has been frequently recorded for shade plants (e.g. Montfort & Neydel, 1928, p. 812), and those aquatic plants which are also adapted to low light intensities (e.g. *Elodea*: Ruttner, 1926, pp. 14 and 21; green algae: Juday & Schomer, 1935, p. 79; Curtis & Juday, 1937, p. 125; and diatoms: Gaarder & Gran, 1927, p. 37, Table 6; Marshall & Orr, 1928, p. 325; and Jenkin, 1930, p. 34). The nature of the inhibition and its relation to the intensity of the light does not seem to have been fully elucidated; but it is probable that several factors may be involved, including the rearrangement or contraction (systrophe) of chloroplasts, the accumulation of end-products (Schreiber, 1927, p. 12), and death of increasing numbers of cells after prolonged exposure to very bright light.

The results on July 25 1933 might be accounted for, in part at least, by systrophe; in that case, the contraction of the chloroplasts might be supposed to have begun at an energy flux of about 40 joules or 9·6 g.-cal. per hr., causing a marked fall in the rate of photosynthesis; the contraction would have reached its limit by 60 joules per hr., and would then act as a direct "limiting factor" at all higher illumination intensities, in which the oxygen production remains at a constant level. Similarly, for the other short exposures, although the average light intensity was below the threshold for inducing systrophe, yet the occasional exposures to high intensity that occurred might be sufficient to induce and maintain systrophe as a limiting factor for most of the time.

In any case, interesting results might be expected from a fuller investigation of such material as *Coscinodiscus excentricus*, with its well-marked inhibition at illumination intensities (e.g. 10 g.-cal. per cm.^{2} per hr.) so much below those of full daylight. This species may well be contrasted with the shallow-water diatoms reported by Curtis & Juday (1937, p. 131) as giving their maximum

yield of oxygen at the surface of a freshwater lake, where the energy flux was 60 g.-cal. per cm.^{2} per hr.

Turning from the maximum energy flux, in which photosynthesis can continue, to the minimum leads to a consideration of the "Compensation" value. Here there are really two distinct conceptions to be found in previous work: the first is the conception of an intensity of light, or energy, which is sufficient to maintain the *status quo*, so that the plant's oxygen production by photosynthesis will exactly balance its oxygen consumption by respiration during the few hours of the investigation; the second is the conception of an intensity of light which is economically sound, so that the plant's oxygen production by photosynthesis during the hours of daylight would be sufficient to balance the consumption during the whole 24 hr. The first of these may conveniently be called the "compensation intensity" (Pettersson *et al.* 1934, p. 4) and the second the "compensation point" (Marshall & Orr, 1928, p. 324). It is clear that the illumination at the "compensation point" must be greater than at the "compensation intensity"; yet it is the minimum at which the plant in question could survive in nature, and is still too low to allow for any increase in crop.

The compensation intensity cannot be as accurately derived from the present results as from those of Pettersson and others (1934, p. 4), since the exposures were not designed to that end. The energy in the illumination at the compensation point, however, can be ascertained from the 24 hr. exposures of *Coscinodiscus*. On July 19–20 1934, the bottles, in which compensation occurred in just under 24 hr., received 9 joules per cm.^{2} during the exposure. Since daylight lasted for about 16 hr. out of the 24, the average energy flux at the compensation point was 0·55 joule or 0·13 g.-cal., per cm.^{2} per hr. This is equivalent to the energy in visible daylight of an intensity of about 360 lux.

Pettersson and his colleagues (1934, p. 4) found the compensation intensity for mixed plankton in the Gullmar Fjord to be about 400 lux, and this has since been confirmed by Höglund & Landberg (1936, p. 33). The intensities found by two such different methods as theirs and mine are remarkably similar, when it is remembered that although the compensation intensity is naturally lower than the compensation point, yet their intensity would tend to be higher than mine because their estimation of respiration included that of the animals in the samples as well as that of the phytoplankton.

Schreiber (1927, p. 12) found the compensation point for *Biddulphia mobiliensis* to be as low as 100 lux, using constant artificial light, but he also found the optimum for this species to be no more than 800 lux or about 0·5 g.-cal. These values, however, are not strictly comparable with those obtained in the sea because of the differences in the spectral distribution of the energy in natural and artificial light.

Schomer & Juday (1935, p. 187) found higher intensities of energy at the compensation point for green algae in some Wisconsin lakes, varying from 0·25 to 0·95 g.-cal. per cm.^{2} per hr.

Unfortunately no direct comparison can be made with Clarke & Oster's (1934, p. 72) measurements of light intensity at Wood's Hole and in the Gulf of Maine, since they give their results for certain blue and red regions of the spectrum only.

Further measurements of available submarine energy will almost certainly bring into line the apparently discordant results which have so far been recorded as to the depth at which compensation occurs in the sea; for these depths vary from close to the surface in the Clyde in winter (Marshall & Orr, 1928, p. 341) to 45 m. in the English Channel in summer (Fig. 8) or 50 m. in the Gulf of Maine (Clarke & Oster, 1934, p. 72); probably even greater depths would be found in the very transparent waters of the Mediterranean or open Atlantic. The differences in depth are almost certainly due to differences in transparency and in colour of the water in these different localities, as well as to differences in the intensity of the incident light at the time of the investigation. Marshall & Orr (1928, p. 325) have already pointed out the effect of the lower intensity of winter daylight and of reduced transparency in spring, which they attribute to diatoms, in bringing the depth of compensation nearer to the surface than it is at midsummer. Reduced transparency, due to silt, could easily account for the fact that, in the shallow waters of Frederikshavn, Nielsen (1932, p. 6) found the compensation point for samples of mixed plankton at depths between 4·5 and 7 m. It certainly does not seem necessary to postulate, as he does, that, because Marshall & Orr found the compensation point as deep as 30 m., his plankton was adapted to brighter light than theirs. Just as great differences in the depth of the compensation point are indicated elsewhere; calculating from photo-electric measurements (Atkins & Poole, 1933, fig. 7, p. 147), the energy at 20 m. in the open water of the English Channel, for instance, is twice that in the inshore waters of Whitsand Bay; and in the Gulf of Maine the transparency is three times that of Wood's Hole Harbour (Clarke & Oster, 1934, p. 72).

Changes in the depth at which the compensation intensity occurs, following changes in the intensity of the incident daylight, were well shown by Pettersson and others (1934, p. 14), for in an extreme case they had to move their samples from 19 m. to the surface (April 17) in order to keep them at a light intensity of 400 lux throughout the exposure. Incidentally, the colour of the illumination would inevitably change considerably between 19 m. and the surface (cf. Fig. 1), so that their plankton was not exposed to similar illumination throughout the time, even if the energy flux were maintained constant.

A rough estimate of the rate of growth of *Coscinodiscus* under optimum light conditions may be gained from the results of the 24 hr. exposures. The optimum energy flux occurred at about 10 m. below the surface, and amounted, on the average, to 13 joules or 3·1 g.-cal. per cm.[2] per hr., during the hours of daylight. In the 24 hr. on July 12–13 the number of cells per unit volume increased from 95 ($\pm$3) to 120 ($\pm$4); this was equivalent to a 25 %

increase in crop (Fig. 8). This estimate is confirmed by analyses of the phosphorus in the diatoms showing an increase from 80 to 100 μg. per million cells (Cooper, Appendix to this paper, Table A).

Analyses of samples of the same culture of diatoms as that used on July 12 (see Appendix) gave 0·08 mg. phosphorus and 0·44 mg. nitrogen per million cells; the oxygen production for a million cells, corresponding to the 25 % increase, was 10–12 ml.; thence, assuming a 1 : 1 ratio between oxygen produced and carbon dioxide assimilated, it may be calculated that the original million cells contained about 2·5 mg. carbon. This gives a ratio for C : P : N of 32 : 1 : 5·5, which may be compared with a typical analysis for fresh* diatoms by Brandt & Raben (1919–22, p. 208) giving a ratio for C : P : N : Fe of 33 : 1 : 5·6 : 2·0.

The ratio of iron to phosphorus in the present cultures was according to Cooper (Appendix, p. 343) at most 1·3 : 1.

An interesting speculation on crop production in the sea in relation to seasonal changes in illumination has also been based on the results of my 24 hr. exposures by Harvey, Cooper, Lebour & Russell (1935, p. 412). They point out that in winter the average amount of light reaching the surface of the sea in a day would be about one-ninth of that in a summer day, so that the total energy available in the sea in the winter would be equal to that below 16 m. in the summer exposure (Fig. 8); there should therefore be no inhibition of photosynthesis in the winter. In fact, allowing even for the effect of winter temperatures, the oxygen production by diatoms behaving like *Coscinodiscus* should only be reduced to one-fourth by the reduction of light to one-ninth. Moreover, there would be less limitation of the natural crop production by shortage of nutrient materials in the sea in winter and early spring, so that the production should be proportionately higher than would be expected from a consideration of the light alone.

There would probably always be a greater shortage of nutrient materials in the sea, than under the experimental conditions, so that the picture of the possible productivity derivable from the present results could rarely, if ever, be realized in nature. Nevertheless, the results serve to show, for *Coscinodiscus*, the nature and extent of the limitations that are set upon photosynthesis by the light in the natural environment.

Some experiments were also made with *Biddulphia regia*, which was found to behave in just the same way as *Coscinodiscus* and to have a similar optimal illumination intensity. Both these species, however, were being used for experiments in the summer, whereas, normally, they are only abundant in the sea in winter or early spring. It is obviously desirable, therefore, to compare the behaviour of such species with that of some summer diatom; the only relevant information for marine diatoms seems to be the observation of Marshall & Orr (1928, p. 337) that the optimum light intensity was the same

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* Their analyses on samples preserved in alcohol are not reliable owing to the loss of phospholipins in solution.

for a summer species of *Chaetoceros* as for the winter species, *Coscinoscira polychorda*, with which they carried out the majority of their experiments.

In conclusion, it may be said that the present method seems unlikely to yield much further information, at least in a place like Plymouth. It is extremely laborious; the distance of the open water from the Laboratory makes it slow; and the exposure to the Atlantic reduces the accuracy of the work done on board ship, as well as the accuracy of the depth at which the samples are suspended. In addition to these specific difficulties there remains the natural variability of daylight, which precludes, at least in the English climate, any really accurate results such as are obtainable under laboratory conditions.

The greatest scope for future work would seem to lie in the laboratory, where the diatoms could be exposed to constant light and temperature: then it should be possible to compare the oxygen production due to light of equal energy content but of different wave-lengths, and also to compare the productivity of different species of diatoms exposed to similar lighting.

Coscinodiscus excentricus has many characters which should recommend it for further work; its symmetrical and compact form and the ease with which it grows under laboratory conditions have already been referred to. In common with other diatoms it presumably shows the colour adaptations which require further elucidation. Last, but not least, it can carry out its photosynthesis in light of very low intensity; it should therefore be possible to supply it with light of sufficient intensity in quite restricted regions of the spectrum, if not actually with monochromatic light, in order to compare the efficiency of radiant energy of different wave-lengths, and to investigate the nature of the inhibition of photosynthesis by relatively bright light.

SUMMARY

1. A method, due to Poole & Atkins, is described for computing the total available energy at any depth in the sea, in joules or g.-cal.

2. The oxygen production of pure cultures of the diatom *Coscinodiscus excentricus* Ehr. was measured by the Winkler method after exposure of the diatoms in bottles at known depths in the sea.

3. The results of simultaneous measurements of oxygen production and of energy are given for 6 days in July and August 1933 and 1934.

4. Factors, other than light, that might affect the rate of oxygen production in the experiments are discussed and shown to be negligible. It is assumed that diatoms can use energy for photosynthesis equally well in all parts of the visible spectrum.

5. When the energy flux during the exposures is less than 7·5 joules or 1·8 g.-cal. per cm.^{2} per hr., the oxygen production is directly proportional to the energy. The utilization of the available energy then amounts to about 7 %.

6. When the energy flux is greater than 7·5 joules per cm.^{2} per hr. the oxygen production is gradually inhibited, but the results are less consistent. The nature of this inhibition, and the causes of the irregularity in the results are discussed. Systrophe appears to be induced by an energy flux of about 40 joules, or 9·6 g.-cal., per cm.^{2} per hr.

7. The energy flux at the "compensation point" is found to be 0·55 joule or 0·13 g.-cal. per cm.^{2} per hr. This is compared with the results of previous workers.

8. Compensation in the clear, open water of the English Channel in summer occurred at a depth of about 45 m.

9. Analyses of the diatoms, based partly on the data in Cooper's Appendix (p. 343), gave a ratio of C : P : N of 32 : 1 : 5·5. The ratio for Fe : P was about 1 : 1.

10. Similar results were obtained with *Biddulphia regia* M. Schultze.

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APPENDIX

DETERMINATIONS OF THE PHOSPHORUS AND NITROGEN IN *COSCINODISCUS EXCENTRICUS* EHR.

By L. H. N. Cooper, Ph.D., F.I.C.

Sufficient of the culture grown as described by Miss Jenkin was placed in a stone jar and mixed thoroughly by aeration. Aliquot parts were syphoned off and filtered through a silk disk having 200 meshes to the linear inch. Since part of the culture passed through the disk, counts were made on the medium both before and after filtration, the difference giving the number of diatoms retained by the filter and analysed

for phosphorus and iron and nitrogen as described elsewhere (Cooper, 1934,[*] p. 755; 1935[†], p. 427). The experimental data are recorded in Table A. The sampling procedure was not entirely satisfactory in dealing with culture 81 J. 13, and the concordance between duplicates is not as good as could be wished. As a weighted mean value one million cells contain 82 µg. P and 490 µg. N; but, since the cell volume in different cultures varies considerably, the best basis on which to express results is per ml. of cell matter. Each ml. of cell matter contains 23 µg. P and 120 µg. N. The ratio of nitrogen to phosphorus shown by the last two experiments is 5·5 when each is expressed in grams weight, or 12·2 when expressed as milligram-atoms.

TABLE A. ANALYSES OF *COSCINODISCUS EXCENTRICUS*

Culture	10⁶ cells contain			Numerical ratio N : P	Ratio Fe : P	Volume of 10⁶ cells in ml.	1 ml. of cell matter contains	
	P µg.	N µg.	Fe µg.				P µg.	N µg.
81 J. 13	79	611[1]	66	7·7	0·9	3·7	21·3	165[1]
	98	748[1]	124	—	1·3	—	26·5	202[1]
83	77	435	36	5·42	0·5	3·9	19·9	112
	—	403	—	—	—	—	—	104
81	80	440	94·5	5·50	1·2	3·3	24·2	133
81, after 24 hr. at sea	*ca.* 100							
Weighted mean	82	490	—	5·5	—	—	23	120

[1] Counts on the filtrates from these samples are lacking and the N content of 10⁶ cells may be high.

The iron analyses are very discordant and at the time this was thought to be due to contamination, which would lead to the ratio of iron to phosphorus being unduly high. Later work (Cooper,[†] 1935, p. 429) on diatom catches taken from the sea showed clearly that the ratio Fe : P in such catches was still higher, about 4 : 1.

Harvey (1937)[‡] has now shown that this 4 : 1 ratio does not measure the relative requirements of iron and phosphorus for growth of diatoms. Much of the iron appears to be adsorbed on their surface as colloidal hydroxide or phosphate. His experiments with *Nitzschia closterium* suggest that the true ratio, on a weight basis, of iron to phosphorus required for growth is 1 : 175 or less. Analyses had shown that 250 mg. P and only 5 mg. Fe or thereabouts per cu. m. had been removed from a culture medium by growing *C. excentricus* (p. 312), giving a ratio of around 1 : 50. These results, inexplicable at the time the experiments were made, fit well with the views now put forward by Harvey and suggest further that the apparent lack of agreement amongst the iron analyses in Table A was due, not to contamination, but to varying amounts of adsorbed iron.

[*] Cooper, L. H. N., 1934. The determination of phosphorus and nitrogen in plankton. *Journ. Mar. Biol. Assoc.*, Vol. XIX, pp. 755–60.

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