Journal of the Marine Biological Association of the United Kingdom Vol 31 No 3 1953

SEASONAL CHANGES IN THE PHYTOPLANKTON DURING THE YEAR 1951–52 AS INDICATED BY SPECTROPHOTOMETRIC CHLOROPHYLL ESTIMATIONS

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(With Text-figs. 1–8)

In a previous paper (Atkins & Parke, 1951) the phytoplankton was studied by the collodion filtration of 20 l. samples, the extraction of the filter disk with 80% aqueous acetone and the comparison of the extracts in a Kober colorimeter against each other and a commercial chlorophyll preparation. The interference of pigments other than chlorophyll was eliminated or much reduced by the use of a Schott RG 1 filter, which we subsequently found had previously been thus used by Rohde (1948). There was, however, frequently a delay in the filtration of our large samples and an increase in accuracy was desirable.

For the present work we were fortunate in having a Unicam spectrophotometer with 4·0 cm. rectangular glass vessel for which 10 ml. of extract sufficed. This was prepared usually the day after the water had been collected, and 1 or 2 l. of water were sufficient, according to season. Unfortunately, the 4 cm. vessels got broken, so for a time 1·0 cm. vessels were used and the results obtained for March and April were necessarily less accurate. The measurements of percentage transmission, as adjusted against a blank with solvent, showed a strong absorption in the red and an even stronger one in the blue-violet. Results were, however, based on the minimum transmission in the red, since the shorter wave-absorption band was frequently masked by a general absorption occasioned by carotins and xanthophylls. The accuracy of the spectrophotometer was checked at 6563 and 4861 Å. and found to be exact, using the hydrogen lamp.

The Spectral Absorption Curves

In Fig. 1 the maximum absorption percentage of the red absorption band at 635 mμ is plotted against the concentration of the commercial chlorophyll in mg./l. The relation is strictly rectilinear up to 4·0 mg./l., and by 8·0 has fallen to 31·8% against 35·8 for rectilinearity. It would appear from the position of the band maximum that the preparation contained both chlorophyll *a* and *b*. Fig. 2 shows the absorption curves of two Chlorophyceae, in pure culture, for which we are indebted to Dr M. Parke. That for *Chlorella* I (by F. Gross) shows the chlorophyll absorption bands with minimum transmissions at 655 and 420 mμ, but for *Chlamydomonas* III (by Mrs Foyn), a solution of greater

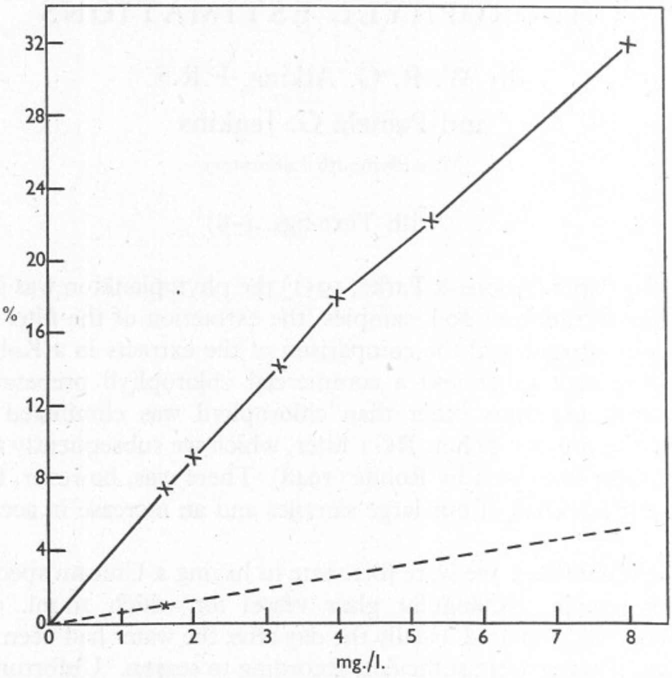

Fig. 1. Percentage absorption at 635 mμ (the position of minimum transmission in the red) for an 80 % aqueous acetone solution of a dry commercial chlorophyll the concentrations of which are given on the abscissae in mg./l., equivalent to 10 mg./m.^{3} when using 10 ml. plankton extract from 1 l. of sea water.

density also has a minimum at 655 mμ but shows complete absorption before the 420 mμ band has been reached. The same is shown by a strong solution, 320 mg./l. of the commercial chlorophyll, which gave maximum absorption at 640 mμ.

In Fig. 3 is shown an extract of *Hemiselmis rufescens*, with sharp absorption maxima at 655 and 435 mμ, also the commercial chlorophyll, 16 mg./l., showing maxima at 635 and 410 mμ. The characteristic buff colour of the alga is due to the presence of a water-soluble crimson, which remains on the collodion or paper and is not dissolved by the aqueous acetone. The wider bands exhibited by the chlorophyll solution are probably due to admixture of the two forms.

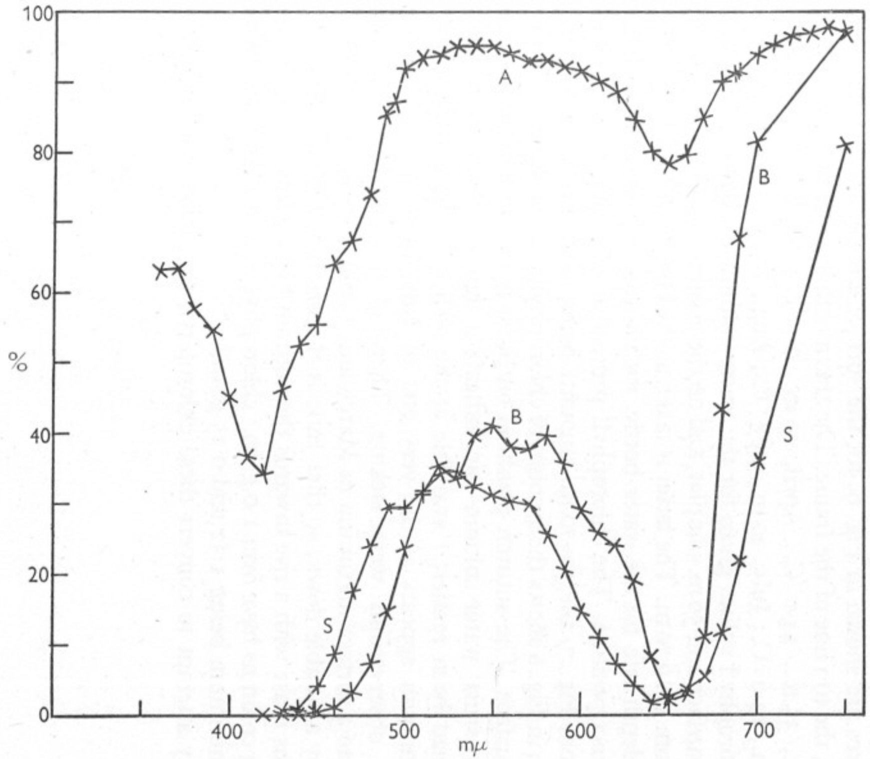

Fig. 2. Percentage transmissions from: S, a stock solution of a dry commercial chlorophyll in acetone, 90 %, with water; A, an 80 % acetone extract of a culture of *Chlorella* I; and B, the same of *Chlamydomonas* III. The abscissae in this and the three following figures show wave-lengths in millimicrons.

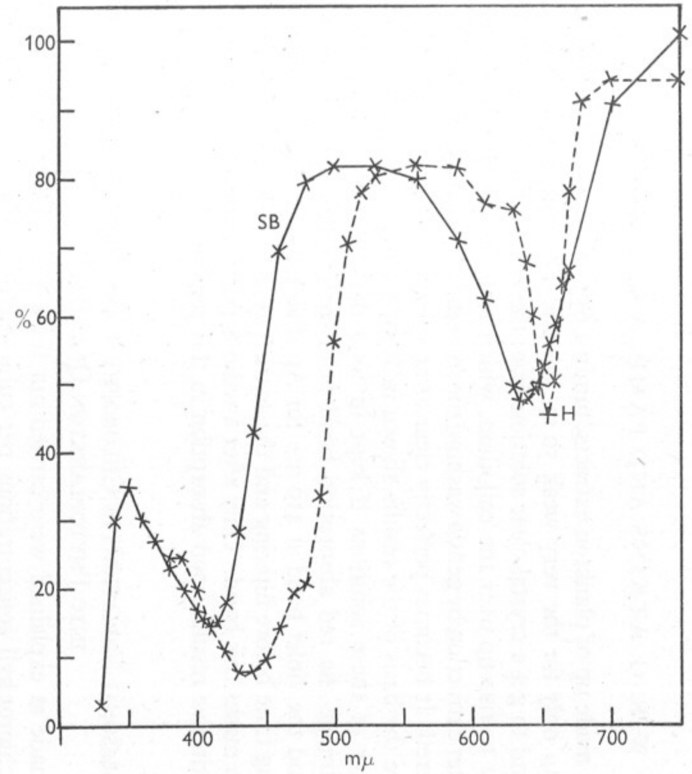

Fig. 3. Percentage transmissions from: SB, an S/20 dilution of the chlorophyll; H, an 80 % acetone extract of a growth of *Hemiselmis rufescens* Parke, developed from water of station E1, 50 m., 7 January 1952 with a very little *Nitzschia closterium*.

Fig. 4 is made up of plankton extracts, minima 655 and 435 mμ for surface, and 650 mμ only for the very weak 50 m. sample. To avoid turbidity from fine clay and to get a crystal clear solution from the extract it is best to allow the solvent to rise up over the collodion, which rests on the side of a small beaker. After extraction by gentle washing the solvent is drained back into a tube and stoppered. It becomes perfectly clear after a few hours in the dark. Such extracts are the basis of the results shown in Figs. 6 and 7. Fig. 5 illustrates the stability of these solutions if kept in total darkness; after more than 3 years' storage the red absorption band is in each clearly a maximum at 650 mμ, and the violet band at 410 mμ for April and June. The July sample stood a long time before filtering and the extract is obviously of a yellowish tint, which masks this band. Only after a tedious purification is it possible to base quantitative results upon absorption in this region.

The Seasonal Variations in Chlorophyll and their Conversion into Phytoplankton Quantities

Extracts made as explained were carried out for a year, and the results are shown in chlorophyll concentrations per cubic metre in Fig. 6, surface and 50 m., and at intermediate depths when a thermocline existed. These desirable samples were unfortunately not taken in the isothermal March water. The sea temperatures are shown in Fig. 6, for the surface above, and for the bottom just below, the top line of the frame. The thermoclines were found as follows: September, 35 m., 14·0° C.; April, 10 m., 9·7° C.; May, 10 m., 11·7° C.; June, 15 m., 14·0° C.; July, 20 m., 15·8° C.; Aug., 20 m., 15·6° C.

The chlorophyll values given for the water column are based on the mean values for surface and 50 m. samples, and on the assumption that the water was uniform from 50 to 70 m. The latter is taken as the bottom at station E 1, but is actually a depth safe for the water-bottle, with bottom 72–74 m. according to tide and exact position. The chlorophyll present at intermediate depths was read off from Fig. 7, the 50–70 m. amounts being calculated as before. The broken line in Fig. 6 shows the amount of chlorophyll in the column per square metre of surface. The autumn plankton outburst is in late September in an almost isothermal water column—an indication that the deep water mineral nutrients had been rendered available at the well illuminated depths. The winter minimum appears at the very end of November, possibly because December observations were lacking. There is then a rise, steep after February, to a surface maximum in March and a column maximum in April, followed by a tumble down, so that June is the minimum for the year. The observations cease with a rise towards the autumn high. Thus the September and March columns have over 1·0 g./m.^{2} chlorophyll, the April maximum and the June minimum being 1·32 and 0·15 g./m.^{2}.

One may attempt to convert these chlorophyll quantities into weights of

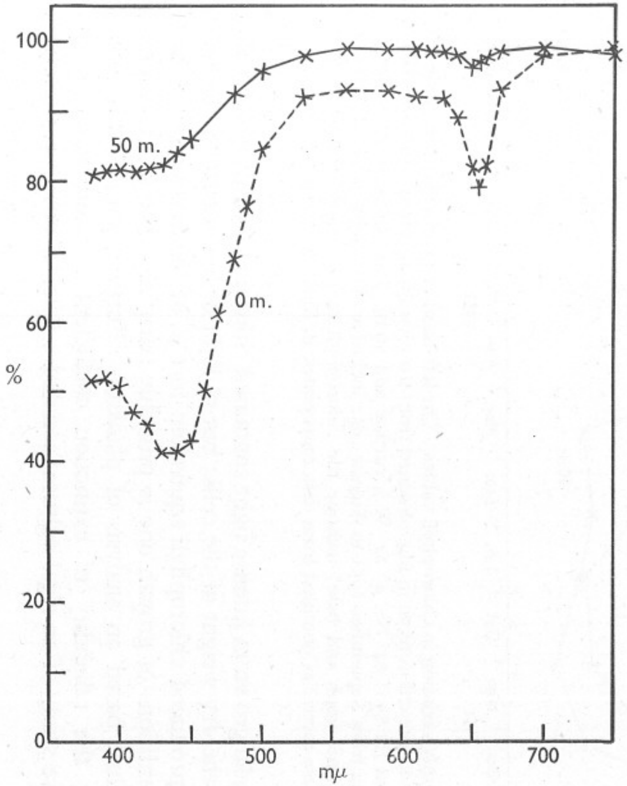

Fig. 4. Percentage transmissions given by extracts from plankton, collodion filtered from 0 and 50 m. 2 l. samples of water from station E1, 11 March 1952.

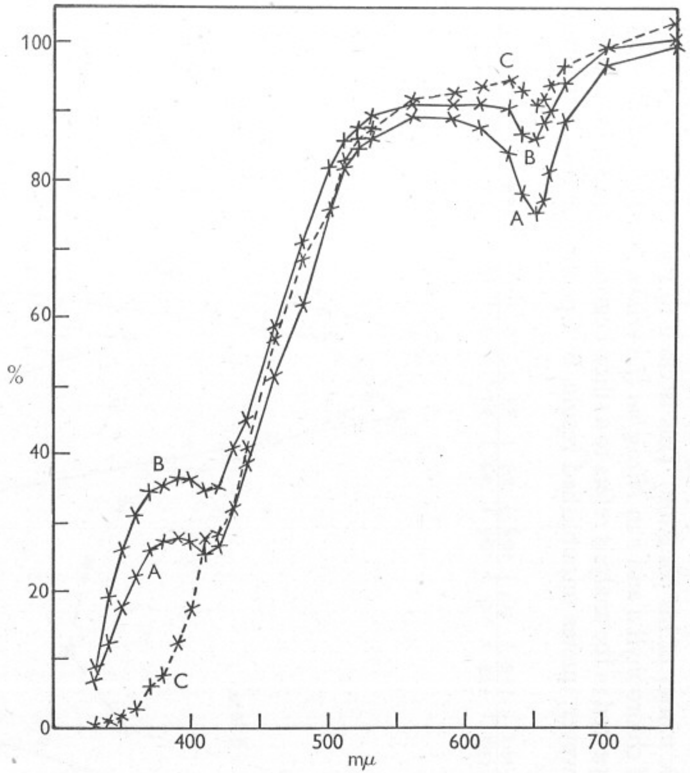

Fig. 5. Percentage transmissions given by extracts qualitatively similar to those of Fig. 4, but from about 20 l. samples through paper using larger volumes of aqueous acetone. Those from station E1, surface, were for April (A), June (B) and July (C), 1949. There was delay in filtering and samples were stored in the dark for over 3 years.

phytoplankton as follows. Riley (1941*a*) found 2·91% of chlorophyll in the dry organic matter of his plankton. This is close to Pace's (1941) 2·32% for the sum of chlorophyll *a* and *b* in *Nitzschia closterium*, in which a lower value is to be expected as the analysis refers to a silica-bearing organism only. Strain (1951), however, quotes unpublished results by Spoehr and Milner showing

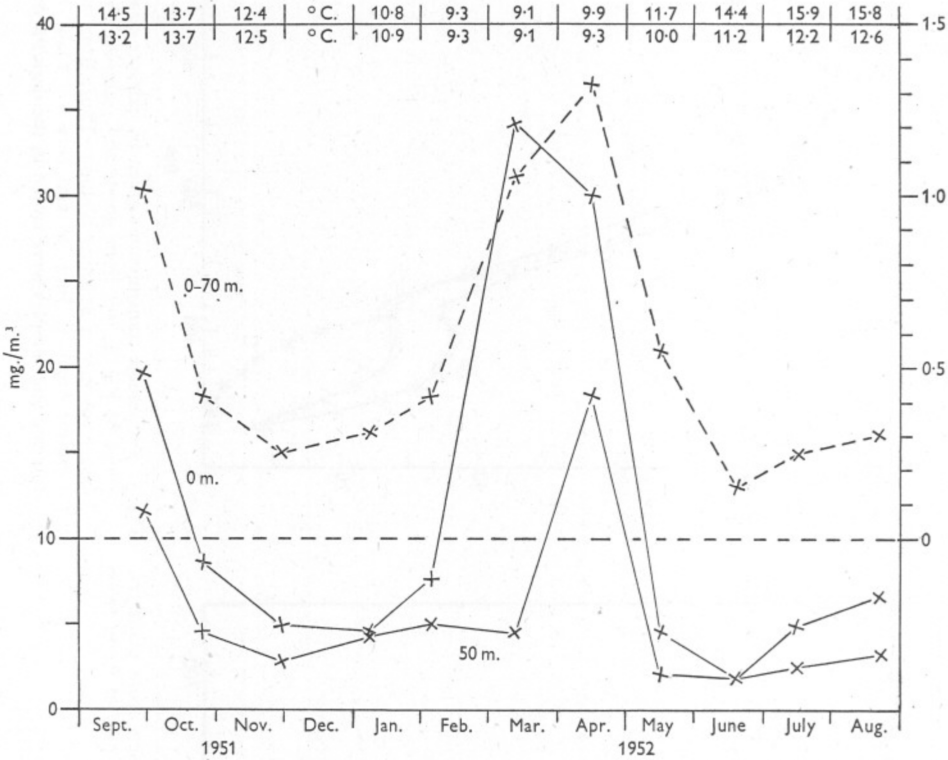

Fig. 6. Monthly variation in chlorophyll values. The left-hand ordinates (for continuous line graphs) denote chlorophyll in mg. obtained from the phytoplankton of 1 m.³ of sea water at station E1 (50° 02′ N., 4° 22′ W.), surface and 50 m. The abscissae show dates of sampling from September 1951 to August 1952 inclusive. The right-hand ordinates (for broken line graph and base) indicate the chlorophyll in the water column, 0–70 m., per square metre, as obtained from data represented in this figure and Fig. 7.

that *Chlorella* grown in intense light contained chlorophyll equivalent to only 0·03% of the dry weight of the cells; but in light of low intensity, the same organism produced chlorophyll equivalent to 6% of the dry weight. Under normal conditions of growth one is probably fairly near the truth in taking Riley's value, based on analysis of plankton. Furthermore, Rohde, when discussing the reliability of extinction coefficient measurements in the quantitative study of an algal culture, concludes with 'if possible, therefore, one should carry out counts of the cell or colony concentration and determinations of the chlorophyll content in parallel with the photometric determinations'. Taking 2·91 % of chlorophyll gives a factor 34·5 by which the weight of dry matter may be found from the pigment. To convert to wet weight the dry weight is assumed to be 20 % (Atkins, 1923), as was done in the calculation of the phytoplankton crop from phosphate consumption. When one adds

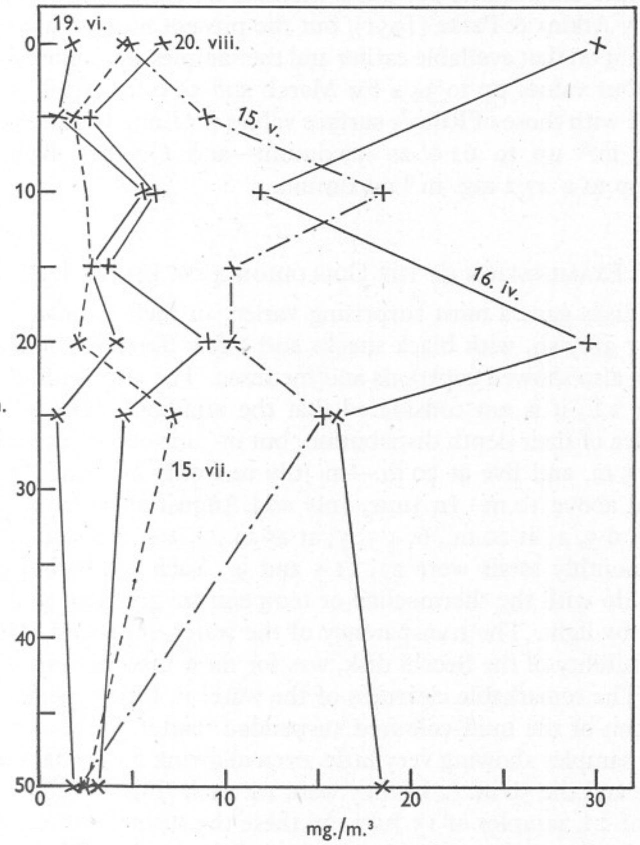

Fig. 7. Variation in chlorophyll values (as mg./m.³) with depth of sampling (metres) on five dates in 1952.

together the wet weight of phytoplankton as found for the 11 months, and for the missing December observation takes the mean of November and January, one arrives at 1·09 g. as the content in the column. This may be compared with the value found from phosphate in 1923, 1·4 g.; and in the less rich water observed more recently Atkins has found for 1948 a consumption, indicating a production, of 84 % of the 1923 value, namely 1·17. The actual quantity of phosphate available in 1948 was only 75 % of the 1923 value. Were the utilization less efficient than in 1948 and proportional to the amount available in winter (for which see the discussion in the accompanying paper on phosphate), the sum of the phytoplankton produced as a minimum value would be 1·05 g. This is close enough to the yearly total of the monthly standing crop to suggest that this must be eaten almost as fast as it is produced.

The surface chlorophyll per cubic metre is on the whole greater than that recorded by Atkins & Parke (1951), but the present method appears to be an improvement on that available earlier and the samples were more expeditiously handled. Our values up to 34·2 for March and 30·0 for April 1952 are quite comparable with those of Riley's surface values for Long Island Sound (1941 *a*) —17·4 mg./m.^{3} up to 62·0 as maximum—and Georges Bank (1941 *b*)— 5·6 mean up to a 27·2 mg./m.^{3} maximum.

Examination of the Collodion 4 cm Filter Disks

The filter disks gave a most surprising variety in their intensity. The colour was usually greyish, with black specks and a few fibres visible with a hand-lens, which also showed copepods and medusae. The volume filtered being so small, 1 or 2 l., it is not considered that the numbers observed can give an accurate idea of their depth distribution, but in June one medusa per litre was found at 15 m. and five at 20 m.—in July one only at 15 m. No copepods were found above 10 m. In June, July and August at 10 m. we got 1, 0, 1; at 15 m., 7, 0·5, 2; at 20 m., 6, 4·5, 5; at 25 m., 8, 3·5, 0·5; and at 50 m., 5, 3, 0·5. The monthly totals were 27, 11·5 and 9. Such a distribution can have nothing to do with the thermocline or temperature gradient, and is probably influenced by light. The transparency of the water, indicated roughly by the depth of visibility of the Secchi disk, was for these months respectively 23, 18 and 11 m. The remarkable clearness of the water in June is perhaps related to the collection of the mud-coloured suspended matter in the surface sample, the deeper samples showing very little, even allowing for the fact that save for the surface and the 50 m. (2 l.) they were 1 l. each (Fig. 8). Fig. 8 also shows the series of 2 l. samples of 15 July. In these the surface and 50 m. are rather similar, and 25 m. shows up as darkest in the photograph. Visually it was not so but had a yellowish tint, as had also 50 m. to a lesser extent.

The Botanical Composition of the Phytoplankton at Station E 1

This station has been visited for many years and records of its phytoplankton have been published in the *Bulletin Planktonique* up to 1912 and summarized later by Ostenfeld (1931) but the following observations may be of interest. E 1 is 10 miles S.W. of the Eddystone and the list published by Harvey,

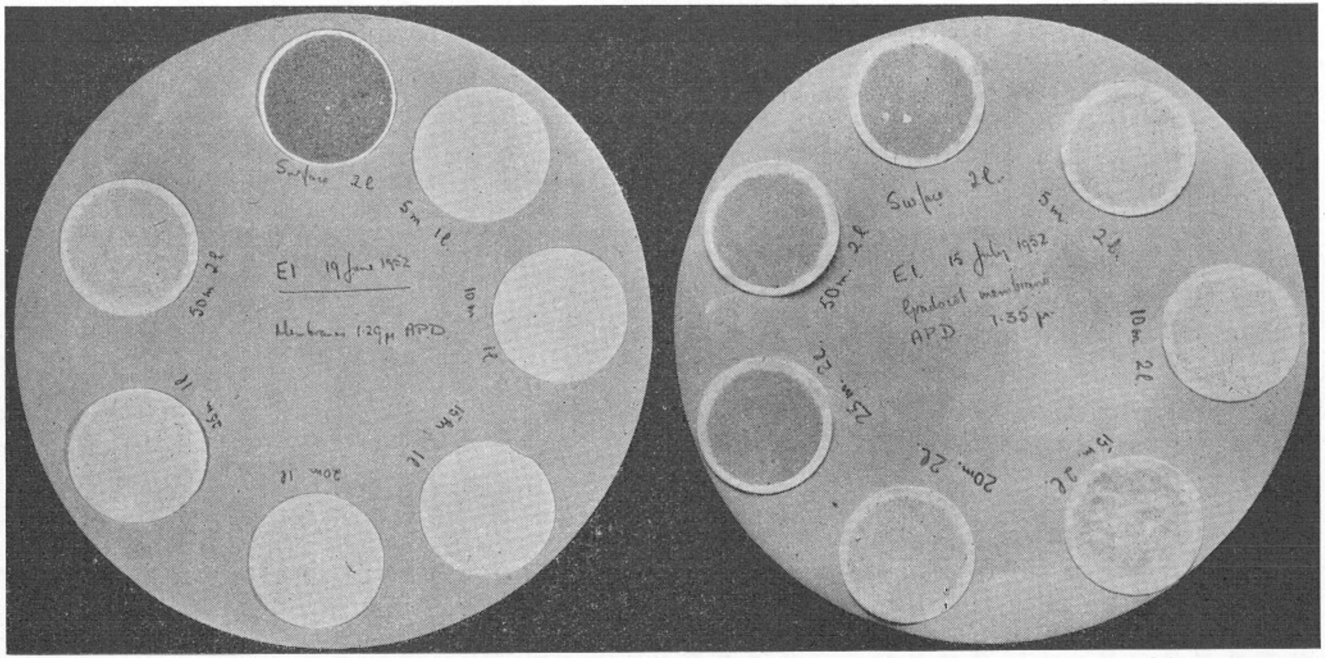

Fig. 8. View of the collodion filter disks with collected matter. Series for 19 June (left) and 15 July 1952 (right).

Cooper, Lebour & Russell (1936) concerns station L4, depth 50 m. and 14 miles nearer shore, about $5\frac{1}{2}$ miles from Plymouth breakwater. We had no plankton hauls and no time to count the algae in the filter disks, but a qualitative examination was made as follows. Samples of the water, 100 or 250 ml., were placed in conical flasks enriched with Miquel's solution and exposed in a south window in winter or a north window in late spring and summer. Some, but probably not all, of the phytoplankton multiplied rapidly, and periodical examinations were made. The plus signs in Tables I and II are an attempt to indicate the relative amounts found in the earlier stages of the cultures; +, present; + +, more frequent; + + +, plentiful; + + + +, very plentiful. Though sampling was begun in September it was only, unfortunately, on 8 February 1952 that the enriched samples were exposed in the window; before that the Winchester bottles had diffuse light in a south room. The earlier dates are probably too sparsely represented. No attempt has been made as yet to represent the depth distribution.

Table I shows the Bacillariophyceae found, twenty species, of which *Nitzschia closterium*, *Melosira Borreri* and *Navicula* sp. were the most regular in occurrence. As compared with the L4 list of 1935 it is remarkable that *Nitzschia closterium* then appeared in negligible amount in March, April and September. *N. delicatissima* and *N. seriata* appear on both lists. *Paralia sulcata*, common at L4, was only found once at E1. *Guinardia flaccida*, which occurred regularly at L4, was absent from E1, as were also over sixteen other species. Found at E1, but not mentioned for L4, were *Fragilaria* sp., *Grammatophora marina*, *Melosira* sp., *Rhizosolenia alata* f. *genuina* and *R. hebetata* f. *semispina*.

Table II lists the Chlorophyceae, Chrysophyceae, Cryptophyceae, Dinophyceae and Miscellaneous found as for Table I. None of the Chlorophyceae are recorded in the L4 list, yet in our cultures they were the striking feature in most of the April—August samples, save the 0 m. samples, the 5 m. for April and the 50 m. sample for July, which were brown with diatoms; also, the 20 m. July sample had a very minute bacterium (with one flagellum visible) which produced a pink colour, modified to buff by a Cryptomonad. This organism became conspicuous in the 10 m. sample later. In several of the series 20 m. was noticeably behind the others in development.

Of the Chrysophyceae, *Phaeocystis* was recorded from L4 and has often been found at E1, but it was absent from our samples. Two others, however, were present at E1 though not at L4: *Coccolithophora* sp., very plentiful in July and August samples, and a member of the Chrysomonadaceae.

The Cryptophyceae afforded two records, in July a species of the Cryptomonadaceae and most remarkably from 50 m., of 7 January, we obtained an almost pure growth of *Hemiselmis rufescens* Parke, which was identified by its appearance and the scarlet pigment insoluble in 80% acetone which was left on the collodion. Its chlorophyll with possibly other pigments gave the curve

TABLE I. ALGAE IDENTIFIED IN CHEMICALLY ENRICHED SAMPLES.

BACILLARIOPHYCEAE:	27. ix. 51	24. x. 51	7. i. 52	5. ii. 52	11. iii. 52	16. iv. 52	15. v. 52	19. vi. 52	15. vii. 52	20. viii. 52
Asterionella japonica Cleve & Möller*	.	.	.	.	++	.	++	.	.	++
Cerataulina Bergonii H. Pérag.†	.	.	.	.	.	.	.	.	.	+
Chaetoceros sp.	.	.	++	.	.	.	.	.	+++++	.
Coscinodiscus sp.	+	.	.	.	++	.	.	.	.	.
Fragilaria sp.	.	.	.	.	.	++	+	.	+	.
F. oceanica Cleve	.	.	.	.	.	.	.	.	.	++
Grammatophora marina Kütz.	.	.	.	.	.	.	.	.	.	+
Leptocylindricus danicus Cleve	.	.	.	.	.	.	.	.	+++	++
Melosira borreri Greville	++	++	.	.	++	++++	+++	++	.	+
Navicula sp.	.	.	.	++	+++	++++	++++	++++	+++	++
Nitzschia closterium Ehr.	+++	+++	++++	+++	+++	++++	+	+++	++++	+++
N. delicatissima Cleve*	.	.	.	.	.	.	.	.	.	++++
N. seriata Cleve†	.	.	.	.	.	.	.	.	.	++
Paralia sulcata Ehr.‡	.	.	.	++	.	.	.	.	.	.
Rhizosolenia alata Brightw. f. *genuina* Gran.‡	.	.	.	.	.	.	.	.	+	+++
R. f. *indica* Péragallo	.	.	.	.	.	.	.	.	.	+
R. hebetata (Bail.) f. *semispina* Hensen‡	.	.	.	.	.	.	.	.	.	++
Skeletonema costatum Greville‡	.	.	.	.	++	.	+	.	++	+++
Thalassiosira gravida Cleve	.	.	++	.	+++	++	.	.	.	.
Thalassiothrix (*Thalassionema*) *Nitzschiodes* Grun.‡	.	.	.	.	.	.	.	.	.	+

* For southern part of North Sea or Flemish area.
† Indicates species similarly listed for eastern part.
‡ Indicates species listed by Ostenfeld (1931) as common in western part of English Channel.

September, October, January and February samples enriched and exposed in window, 8 February 1952; the same holds for Table II.

TABLE II. ALGAE IDENTIFIED IN CHEMICALLY ENRICHED SAMPLES.

	27. ix. 51	7. i. 52	5. ii. 52	11. iii. 52	16. iv. 52	15. v. 52	19. vi. 52	15. vii. 52	20. viii. 52
CHLOROPHYCEAE:									
Chlamydomonas sp.	.	.	.	++	.	.	.	.	.
Chorella sp.	.	.	.	++++	++++	++++	+++	++	+
Endoderma sp.	.	.	.	+++	++++	+	+++	.	.
Halosphaera viridis Schmitz	+	.	.	.	.	.	.	.	.
Platymonas tetrahele West.	.	.	.	.	.	.	.	++	.
Pyramimonas sp. Schmarda	.	.	.	+++	.	++	.	.	.
Stichococcus bacillaris Naeg.	.	.	.	+++	++++	+++	+++	++	.
Ulothrix subflaccida Wille	++	.	+	.	.	.	.	.	.
CHRYSOPHYCEAE:									
Chrysomonadaceae sp.	.	.	.	.	.	.	.	++	.
Coccolithophora sp.	.	.	.	.	.	.	.	++++	++++
CRYPTOPHYCEAE:									
Cryptomonadaceae sp.	.	.	.	.	.	.	.	++	.
Hemiselmis rufescens Parke	.	++++	.	.	.	.	.	.	.
DINOPHYCEAE:									
Prorocentrum micans Ehr.	.	.	.	.	.	++++	.	.	.
MISCELLANEOUS:									
Flagellates, colourless	.	.	.	.	.	++	.	++++	++++
Amoebae	.	.	.	.	.	.	+++	.	++
Ciliates, colourless	.	.	.	.	.	++	++++	++	++
Bacterium, pink	.	.	.	.	.	.	.	++++	.

shown in Fig. 3. Of the seven Dinophyceae in the L4 list only *Prorocentrum micans* was observed in the E1 cultures.

We desire to express our indebtedness to Miss D. Ballantine, Dr M. V. Lebour, Dr T. J. Hart and Dr M. Parke for much help in the identifications, also our thanks to Mr F. A. J. Armstrong for filtering the samples and for temperature observations and to Mr A. E. Stoate and Mr A. Mattacola for the photographs. Finally, for these and many other sea-water samples and Secchi disk observations we have pleasure in thanking Lieut.-Comdr. C. A. Hoodless, D.S.C., R.N.R., and the crew of the R.V. *Sabella*.

Summary

Chlorophyll from the phytoplankton at station E1 was examined from September 1951 till August 1952, from 0 to 50 m. The minimum was in June, 1·8 mg./m.^{3}, and the maximum 34·2 in March, both surface samples. The maximum 50 m. sample contained 18·4 mg./m.^{3} in April. The winter minima were 4·6 and 4·3, surface and bottom, in November. Converted to water column (70 m.) values, over 1·0 g./m.^{2} is obtained for the wet weight of phytoplankton for September and March, the April maximum and the June minimum being 1·32 and 0·15 g./m.^{2}. A comparison with the production obtained from phosphate analyses suggests that the phytoplankton crop each month is rapidly devoured.

Exact proportionality was found between concentration and absorption in a chlorophyll band up to 4·0 mg./l. and a moderate error up to 8·0 mg./l. The spectral absorption curves have been given for pure cultures of phytoplankton and for cells filtered out of sea water. The chlorophyll in such extracts, made with 80% acetone, is stable when kept in total darkness.

The collodion disks containing suspended clay and the algal cells may show a surprising variation in their colour intensity, from dark grey to a very faint tint, even though obtained from sea water about 20 miles from land and over 70 m. in depth. The surface may be far darker than 5, 10 or 15 m. samples.

The botanical composition of the water was studied by allowing the algae to multiply in diffuse light after enriching the water chemically. Eight species of Chlorophyceae, and one species each of the Chrysophyceae and Dinophyceae were recorded. Of the Cryptophyceae one winter sample from 50 m. gave a nearly pure growth of *Hemiselmis rufescens* Parke. The diatoms *Melosira borreri*, *Nitzschia closterium* and *Navicula* sp. occurred commonly.

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