On the Rate of Diatom Growth.

By

H. W. Harvey, M.A.,

Hydrographer at the Plymouth Laboratory.

With 3 Figures in the Text.

THE series of events taking place in the sea which influence the growth of phytoplankton, and consequently the general fertility, has provided a fruitful study during recent years. Intensive surveys have been made since simple and sufficiently accurate methods of determining minute traces of phosphates and nitrates in sea-water became available. Over the greater part of the oceans the annual crop of phytoplankton seems to be limited by the quantity of these nutrient salts which arrive annually in the upper layers from below. This quantity depends upon vertical mixing, since nitrogen salts and phosphates are re-formed but slowly from dead organisms, which sink meanwhile to enrich the deep layers. Although this hypothesis gives a simple explanation why some areas are fertile, supporting a rich growth of phytoplankton and others not so much so, and the facts so far ascertained fit well with the explanation, recent investigations in the Southern Ocean (**1**, **2**) indicate that other causes limit the annual production there. The phosphates and nitrates are not fully utilised during the summer, whereas in equally high and higher latitudes in the North Atlantic they are, and, moreover, growth may be very rapid.

A wealth of observations has been collected in widely distant seas, and the questions, often rather nebulous, which have arisen from these require consideration in any attempt to envisage the skein of possible factors which regulate the growth of diatoms in various places.

Purely mechanical means by which phytoplankton organisms are transported vertically may play a leading rôle. Off the west coast of Norway (**3**a, **4**) it has been found that the spring outburst of diatom growth starts around the edges of the fiords and over the edge of the continental shelf, then extending across the 30 miles of sea between these foci; later it commences in the Atlantic water beyond. In the Gulf of Maine (**5**) the spring outburst starts close inshore and over the offshore banks. Between Russia and the Northern ice (**6**) growth starts first

inshore and in the cold Arctic water which has originated from comparatively shallow areas, later in the tongues of warmer Atlantic water which penetrate eastward into the Arctic water. It has been suggested that water near the surface close inshore and over banks where there is upwelling is better seeded with diatoms and their spores, these having been kept better in suspension throughout the winter by turbulence than in the deep ocean. Another likely locus (**3**b) where the surface layers are well seeded at the beginning of sunny weather is close to ice, where the run off remains near the surface and may often contain diatoms which have been frozen in at the end of the previous summer.

Vertical transport in the turbulent water of the open ocean is of necessity greater in those regions where wave motion keeps the water so well mixed that the upper layers do not gain in temperature over the water below. The continuous drain on the phytoplankton population by being carried below the "compensation level," where there is sufficient light for photosynthesis to exceed respiration, is enhanced in such areas, where vertical mixing is not restrained during the summer months owing to density differences being set up (**3**). Further, the surface waters being kept cold, the rate of photosynthesis is kept low. This may to some extent influence the fertility over wide and turbulent expanses of the Southern Ocean, where owing to the great "fetch" of the waves their mixing effect may extend deeper than in the North Atlantic.

It has recently been found in the seas between Iceland and Greenland (**7**), in the Barents Sea (**8**), and in the English Channel (**9**) that ammonium salts occur at greater concentration in the upper layers than in the deeper water. Although the quantity is always very small, some material amount remains after all or practically all the nitrate has been used up by phytoplankton. Nitrate production, which presumably takes place only by bacterial action, has not yet been found to occur in the upper layers of ocean water, although it occurs readily enough in water in contact with bottom deposits. It is not clear how the plants get a sufficiency of nitrogen salts during the summer months, at least in such places as the upper layers of the English Channel, unless they utilise ammonium as it is being formed. In fact, the numerous and more accurate data now accumulated have added little to our knowledge of the processes involved in the nitrogen cycle in the seas.

Physical conditions may explain, either wholly or in part, a group of phenomena connected with the growth of diatoms in temperate and Northern seas. The date of the vernal outburst of growth in the English Channel and off the Isle of Man has been found to depend largely on the amount of sunshine in the early part of the year, yet in Loch Fyne it may occur a month earlier than in the near-by Loch Striven (**10**). Off the west coast of Norway, in materially higher latitude and colder water, it occurs

earlier than in the English Channel. The date may depend upon the total amount of incident light, of which there are no observations yet available, or upon some other cause not yet recognised.

In general it appears that the growth of diatoms during the summer months in relatively shallow inshore waters is greater than in deeper waters out to sea. Although the amounts of nutrient salts at the beginning of the season may not be very different, in the shallower areas mixing due to tides and occasional strong winds keep the upper water layer better refreshed with these salts throughout the summer. In this connection Gran (**3**) has put forward the interesting theory that drainage from the land contains some growth-promoting factor, which would in part account for the great productivity of coastal waters, and perhaps for the inability of phytoplankton to consume all the available nutrient salts in the Southern Ocean which receives little land drainage compared with the Northern Atlantic. It had been found that the addition of a sterilised water extract of soil to a flask of sea-water containing ample phosphate and nitrate increased the growth of diatoms in it.

Mention should also be made of the successive outbursts of diatom growth in the sea, where one dominant species dies out to be replaced by another, and of how diatoms cultured in flasks tend after a time to die out, particularly if bacteria become numerous.

The experimental investigation of influences affecting the growth of diatoms *in vitro* cannot by itself solve the questions which arise from observations such as these. However, the effect of some chemical factors on the rate of growth lends itself to such investigation, and was undertaken in the hope that further knowledge of the physiology of diatoms may open up new lines of enquiry.

Experimental Methods.

The diatom *Nitzschia closterium* was chosen as it grows well, is persistent, and was obtainable free from flagellates. On the other hand it is a neritic species typical only of inshore waters.

I am indebted to Dr. E. J. Allen for the parent culture, which had in its turn been subcultured over a number of years. Up to the present time a persistent culture of marine diatoms has never been obtained completely free from bacteria. In interpreting these experiments, the possibility has always to be borne in mind that differences in the rate of growth of the Nitzschia may have been caused by changes in the bacterial flora or their secretion into the water. It would seem, however, that the additions of the various substances to the sea-water act directly upon the diatoms, rather than indirectly through the bacteria, but this is, of necessity, a matter of opinion.

In order to follow the rate of growth, six flasks (A, Fig. 1) of equal

volume, 293 c.c., were arranged in a carrier. Each was filled with the same culture and 20 c.c. withdrawn, leaving 273 c.c. of liquid and 20 c.c. of air. To one, acting as control, a trace of mercuric chloride or potassium cyanide had been added. The carrier was placed in a water bath kept at constant temperature, usually 16° C., and the whole continuously shaken to and fro by means of a motor. The shaking was sufficient to bring the gases in solution in the liquid into equilibrium with the 20 c.c. of air within a short time. The upper ends of the gauge tubes B were connected

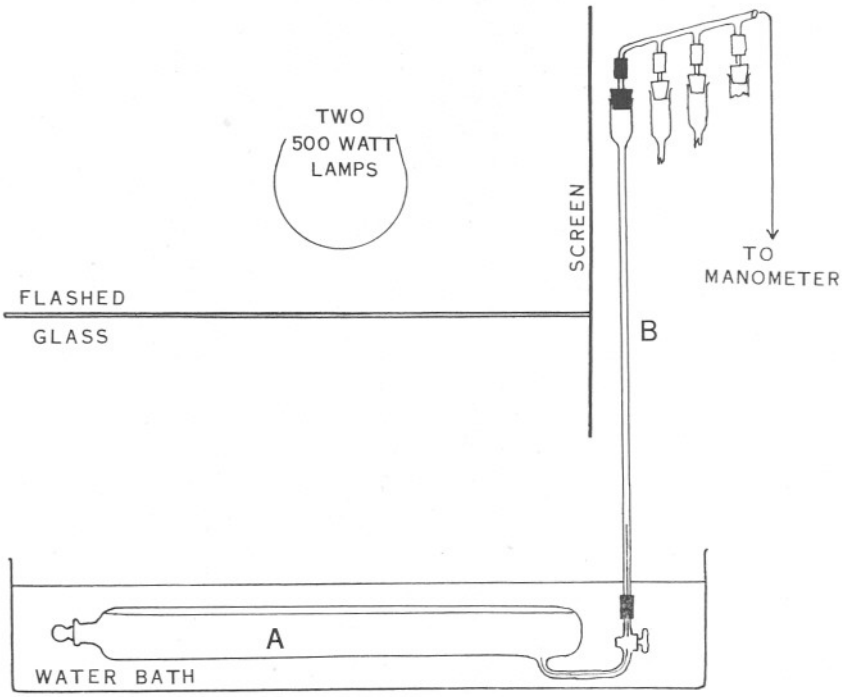

FIG. 1.

to a manometer and the pressure adjusted so that the height of liquid in the gauge tube of the control was at a convenient mark : then the heights in the other tubes were read, the motor being stopped meanwhile. The mark on the gauge tube of the control is so chosen that the 20 c.c. of air in the series of flasks is roughly at normal atmospheric pressure.

Illumination was obtained from two 500-watt gas-filled lamps with the light source 35 cm. above the tubes, and having a sheet of flashed glass immediately below the lamps. This provided more even illumination and reduced the heat radiated to the water bath. On the other hand it reduced the illumination some 50%. After a period of illumination the motor was again stopped, the manometer again adjusted so that the height

of liquid in the control flask gauge was the same as before, thus obliterating the effect of any slight variation in temperature, and the heights in the other tubes read. The increases in height from the previous readings gave the increases in volume of the air above each flask, due to the oxygen given off by the diatoms. This distributes itself between the liquid and the air, increasing the volume of the latter.

The volume of oxygen produced per litre of liquid in each flask, subsequent to the first period of shaking, was calculated in the following manner.

If x c.c. of gas are evolved, mostly oxygen, it increases the volume of the 20 c.c. of air above the sea-water in the flask to $20+x$ c.c., the temperature being 16° C. and the pressure being taken as normal atmospheric pressure, the slight increase due to the rise of water in the gauge tube being neglected. Since the proportion of nitrogen in this air is slightly reduced by the oxygen given off, its partial pressure falls and a little nitrogen is given off from the sea-water. Let a c.c. be the nitrogen and $x-a$ c.c. be the oxygen evolved. The 273 c.c. of sea-water at 16° C. and normal atmospheric pressure in equilibrium with air contains 3·5 c.c. of nitrogen at 16° c. (Fox).

At the beginning the sea-water is in equilibrium with nitrogen at a partial pressure of approximately $\frac{16}{20}$ of 760 mm. Hg. After the evolution of x c.c. of gas it is in equilibrium with a mixture containing $16+a$ c.c. of nitrogen and $4+x-a$ c.c. oxygen, and the partial pressure of nitrogen becomes $\frac{16+a}{20+x}$ of 760 mm. Hg. Hence the volume of nitrogen remaining in the sea water is

$$3{\cdot}5\times\frac{(16+a)\ 20}{(20+x)\ 16}\text{ c.c.}$$

and the loss is

$$3{\cdot}5-\frac{280+17{\cdot}5a}{80+4x}=a$$

From this the gas evolved contains roughly 14% of nitrogen and 86% of oxygen.

The 273 c.c. of sea-water at 16° C. contains when in equilibrium with air at normal pressure 1·74 c.c. of oxygen measured at 16° C. (Fox). After x c.c. of gas have been evolved it is in equilibrium with the $20+x$ c.c. of gas mixture containing $4+0{\cdot}86\ x$ c.c. oxygen. The dissolved oxygen then amounts to $1{\cdot}74\times\frac{5(4+0{\cdot}86x)}{20+x}$ c.c., being an increase of $\frac{5{\cdot}75x}{20+x}$ c.c.

Hence the total oxygen production approximates to $0{\cdot}86x+\frac{5{\cdot}75x}{20+x}$ c.c. measured at 16° C. per 273 c.c. of culture, or $\frac{78x+3x^2}{20+x}$ c.c. per litre measured at N.T.P.

If oxygen is being produced rapidly by the diatoms in the flasks there will be a certain lag in time before it attains equilibrium with the air with which the liquid is being shaken. In order to assess the error likely to arise from this cause the following experiment was carried out.

Two flasks were charged with sea-water approximately saturated with air at 16° C. and two flasks with sea-water which had been partially de-aerated. They were shaken in the water bath for 3 minutes before the first readings of the heights of liquid in the gauge tubes were read. Readings were taken at intervals. In this particular experiment the manometer was disconnected. In order to eliminate a slight change in temperature in the water bath, the readings of flask IV were subtracted from those in the other three flasks, this procedure being comparable with the use of a control flask and manometer in an ordinary experiment.

TABLE I.

CHANGE IN VOLUME OF AIR IN FLASKS LESS CHANGE IN FLASK No. IV.

	I.	II.	III.
After 10 min.	—0·451	—0·483	—0·021 c.c.
20 ,,	·506	·536	·023
30 ,,	·519	·542	·020
40 ,,	·520	·545	·020
50 ,,	·522	·546	·022
80 ,,	·520	·546	·020

The result indicates that equilibrium was quickly approached, and attained within the limit of experimental error in less than 30 minutes. In the above experiment gases were entering the water, while working with diatoms oxygen passes out of the water into the air above.

In many of the experiments qualitative differences in growth rate were observed on keeping the cultures in small flasks in a north window until a thick growth had developed. Visual inspection was sufficient to show any material differences, and this was in many instances confirmed by examination in a Dubosq colorimeter. In some cases counts were made by means of a hæmocytometer having a field of $\frac{1}{10}$ cubic millimeter. In order to obtain representative values it was necessary to count several hundred cells from each culture, or even several thousand when the difference was not very great although clearly seen on visual inspection. I am indebted to Mr. G. M. Spooner for mathematical analysis of several counts.

The effect of Dissolved Phosphate on the Rate of Carbon Fixation by *Nitzschia closterium*.

Nitzschia was seeded into a flask of sea-water which had been passed through a Berkfeld filter and to which an excess of potassium nitrate had been added, equivalent to over 500 mg. of nitrate nitrogen per cubic metre. This was kept in a north window until vigorous growth had used up practically all the dissolved phosphate. Analysis was made difficult by the quantity of diatoms present, but there remained certainly less than 10 mg. P_2O_5 per cubic metre. This culture was then filled into two flasks. The third, fourth, and fifth flasks were filled with the same culture to which 10, 20, and 100 mg. P_2O_5 per cubic metre had been added respectively. The sixth flask, acting as a control, was filled with the same culture to which a little cyanide had been added. Exactly 20 c.c. was withdrawn from each. After standing overnight in the dark they were illuminated and shaken at 15° C., the first reading being taken 45 minutes later.

TABLE II.

Excess of Photosynthesis over Respiration.

c.c. oxygen produced by 1 litre during

	1 hr. 30 min.	2 hr. 25 min.	4 hr. 20 min.	6 hr. 20 min.
Without addition of phosphate . . .	0·12	0·24	0·49	0·67
Ditto . . .	0·15	0·26	0·53	0·75
With 10 mg. per m^3 P_2O_5 added . . .	0·19	0·32	0·66	0·96
20 mg.	0·24	0·41	0·76	1·24
100 mg.	0·25	0·43	0·85	1·26

These values indicate that the addition of phosphate caused an increased rate of photosynthesis as soon as illumination was commenced, having had all night in the dark in which to be absorbed by the diatoms.

With the aim of ascertaining the final increase in fixed carbon due to the addition of phosphate, two experiments were made. Nitzschia was grown in filtered sea-water enriched with nitrate until all, or practically all, the dissolved phosphate had been used up, as shown by analysis. The flasks were filled, the control poisoned with cyanide, 20 c.c. withdrawn from each, and they were brought to constant temperature in the water bath. After a short period of illumination to ascertain that the rate of growth in all was the same within the limits of experimental error, additions of potassium hydrogen phosphate were made to three of the tubes, an equal quantity of liquid being withdrawn. They were then subjected to alternating periods of light and darkness.

Figure 2 shows the rate of growth, that is the excess of photosynthesis over respiration, during illumination. The volumes of oxygen used up in the process of respiration during the periods of darkness do not enter into the values plotted.

It is seen that growth proceeds at a steady slow rate in the tubes having no added phosphate. The increase in rate due to the addition

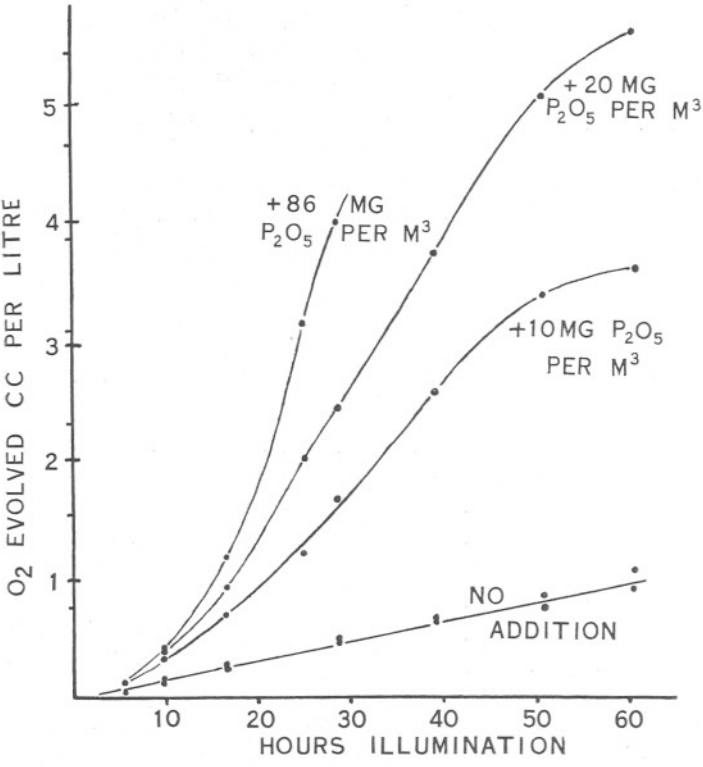

FIG. 2.

of 20 or 86 mg. P_2O_5 per cubic metre remains about the same for 10 hours or more, after which the effect of 86 mg. becomes markedly greater than the effect of 20 mg., although even after 20 to 25 hours only a small part of the 20 mg. has presumably been used up.

The last part of the curve, showing the growth where 10 mg. P_2O_5 per cubic metre had been added, runs parallel with the line or curve showing the growth of the diatoms without added phosphate. This indicates that the 10 mg. per cubic metre had all been utilised and that it gave rise to 2·6 c.c. per litre of oxygen more than in the tubes having no added phosphate. However, the respiration during the periods of darkness was greater

in the flasks with added phosphate, the nett productions of oxygen being as follows :

TABLE III.

NETT OXYGEN PRODUCTION AT THE EXPIRATION OF 60 H. 45 M. LIGHT AND 69 H. 45 M. DARKNESS.

With no addition . . .	0·40 c.c. per litre.
Ditto	$0{\cdot}43_5$,, ,,
With 10 mg. P_2O_5 per m.^{3} added	2·45 ,, ,,

This shows a nett increase of 2·0 c.c. per litre due to the addition of 10 mg. P_2O_5 per m^3.

In the next experiment filtered sea-water with excess of nitrate was seeded with Nitzschia and after some days in a north window filled into five of the flasks, one of which was poisoned with mercuric chloride and used as control. In order to deduce when practically all the phosphate was used up, and to see that all the flasks behaved alike, they were illuminated daily for about 12 hours for four days. The observed increases in volume were 0·445, 0·447, 0·435, 0·440 c.c. respectively, showing remarkably close agreement. To two flasks 10 mg. P_2O_5 per cubic metre was then added, and illumination continued daily for eight days, by which time the rate of growth was the same. The oxygen production, being the nett excess of assimilation over respiration during this eight-day period of alternating light and darkness, was as follows :

Without added phosphate $\left.\begin{matrix}1{\cdot}80\\1{\cdot}50\end{matrix}\right\}$ mean $1{\cdot}6_5$ c.c. per litre.

With 10 mg. P_2O_5 per m.^{3} $\left.\begin{matrix}3{\cdot}9\\3{\cdot}9\end{matrix}\right\}$ mean 3·9 c.c. per litre.

The nett increase in oxygen, due to the addition of 10 mg. P_2O_5 per m.^{3}, is here $2{\cdot}2_5$ c.c. per litre, a value comparable with that of 2·0 c.c. found in the previous experiment.

A peculiar inference arises from these values. The addition of 10 mg. P_2O_5 per cubic metre gave rise to an observed increased growth equivalent to 2·0–$2{\cdot}2_5$ c.c. evolution of oxygen, or assimilation of the same quantity of carbon dioxide per litre, that is to say a fixation of 1·07 to 1·2 gm. of carbon per cubic metre. If the illumination had been continuous there is reason to suppose that this quantity would have been greater, the losses due to respiration during the periods of darkness being eliminated. The ratio of P_2O_5 added to carbon fixed is $\frac{1}{10\cdot7}$ to $\frac{1}{12\cdot0}$; even less if illumination were continuous. There are no analyses showing the ratio of P_2O_5 to carbon in the cells of *Nitzschia closterium*, but Raben's analyses of plankton consisting almost entirely of mixed diatoms gives a ratio of about $\frac{1}{15}$. The addition of phosphate brought about the fixation of seven times the amount of carbon that might have been expected.

The effect of Added Phosphate upon the Rate of Oxygen Consumption by *Nitzschia closterium* in Darkness.

Filtered sea-water, enriched with potassium nitrate, was seeded with *Nitzschia closterium* and kept in a north window until vigorous growth had developed. This was filled into the flasks, to three of which additions of phosphate were made. They were then covered with black cloth and shaken continuously at 16° C. in darkness. The results of this experiment are shown in Figure 3.

A second experiment was made in the same manner, but over a longer

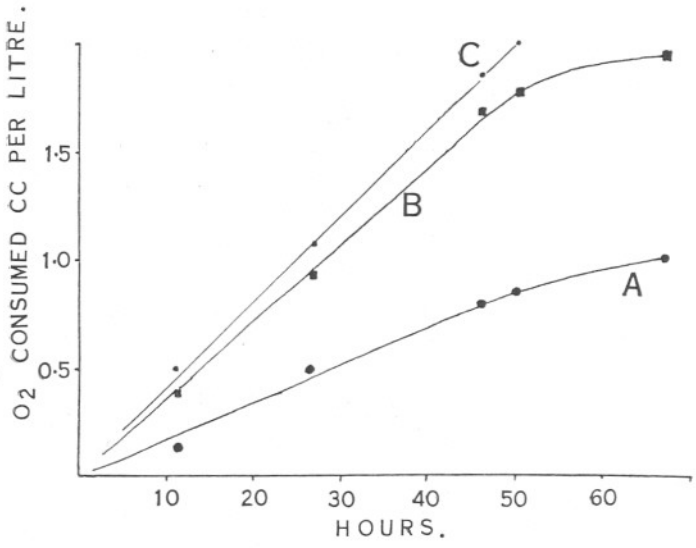

Fig. 3.—Oxygen consumption by Nitzschia in sea-water with excess nitrate in darkness. Curve A shows mean values for two flasks without addition of phosphate. Curve B shows mean values for two flasks with 200 mg. P_2O_5 per cubic metre added. Curve C shows values for flask with addition of 1000 mg. P_2O_5 per m.^{3}.

period of time. As in the previous experiment the oxygen consumption was roughly doubled where 200 mg. P_2O_5 per cubic metre had been added. The increase in rate of oxygen consumption tended to wane about 45 hours after its addition.

In another experiment the flasks were filled with a culture rich in nitrate which had used up practically all the available phosphate. They were exposed to alternating illumination and dim light, not complete darkness in this case. By taking the rate of photosynthesis as equal to the rate of oxygen produced under illumination plus the rate of consumption in dim light, as approximating darkness, the results may be epitomised as follows :

RATE OF PHOTOSYNTHESIS, C.C. O_2 PER LITRE PER HOUR, MEAN OF VALUES FROM TWO FLASKS IN EACH CASE.

	During first day	second day	third day of experiment.
Without addition . .	0·051	0·045	0·041
With 10 mg. P_2O_5 per m.^{3} .	0·081	0·089	0·087
Mean rate of oxygen consumption in dim light :			
Without addition . .	0·008	0·011	0·013
With 10 mg. P_2O_5 per m.^{3}.	0·017	0·022	0·022

From this it appears that the rates both of photosynthesis and of oxygen consumption in darkness are about doubled by providing phosphate even in such small quantity. It is improbable that the increases are wholly due to the increase in quantity of fixed carbon brought about during the periods of illumination, particularly as the first period only gave rise to a very small carbon dioxide assimilation.

Another experiment was made in the same way. Adding 10 mg. P_2O_5 per cubic metre about doubled the rate of oxygen consumption as in the previous experiment. Treating the values in the same way, and making the same assumptions, the rate of photosynthesis increased from nearly double to over treble during the third day. In the previous experiment it remained about double. More phosphate, 86 mg. per cubic metre, rather more than doubled the rate of oxygen consumption and this rate increased as a material quantity of new growth was formed.

Rate of Photosynthesis, c.c. oxygen per litre per hour.	During first day	second day	third day of experiment.
Without addition . .	0·026	·028	·028
With 10 mg. P_2O_5 per m.^{3} .	·048	·078	·10
20 ,, ,, ,, .	·050	·10	·15
86 ,, ,, ,, .	·056	·15	·29

In these experiments the rate of oxygen consumption in darkness is due to respiration by the diatoms and to bacterial and autolytic breakdown of dissolved organic matter. However there is no reason to suppose that the addition of these minute traces of phosphate affects the bacteria and oxidation of dissolved organic matter, and it seems safe to assume that the big differences found in the rate of oxygen consumption are due to the influence of phosphate upon the respiration of the diatoms.

It is considered that they had loaded up with fixed carbon in the form of storage products, which they could not convert into new growing tissue for lack of necessary phosphorus. As soon as this is provided the conversion takes place and in consequence the rate of respiration increases. The following experiments indicate that this conversion is accompanied

by division of many of the cells, and the formation of new pigment, even if the diatoms are kept in the dark.

A culture of Nitzschia in filtered sea-water, heavily enriched with nitrate, was kept for several days in a window until growth had apparently ceased owing to lack of phosphate. This was divided between two flasks, to one of which phosphate was added to the extent of 100 mg. per cubic metre. Both were then kept in darkness for 48 hours. The addition of phosphate resulted in a 29% increase in the number of diatom cells, and the culture was browner in colour than the flask to which no addition had been made.

The experiment was repeated with a fresh culture. The addition of phosphate, after 48 hours in darkness, resulted in a 28% increase in the number of diatom cells. Equal quantities were filtered and the pigment washed out from the diatoms on filter papers by repeated passage of 80% acetone. Colorimeter readings of the two pigment extracts—yellow-green in colour—showed 45% more pigment in the culture to which phosphate had been added.

A consideration of Table II, p. 259, which is typical of several experiments where the culture stood some 18 hours in the dark with added phosphate before being illuminated, is suggestive. Hand in hand with the change of stored material into actively respiring protoplasm, the photosynthetic activity of the cells was increased. During the first hour and a half's illumination the effect of adding phosphate 18 hours previously is marked.

The Effect of Nitrate and of Ammonium upon the Growth of *Nitzschia closterium*.

The results of two experiments with cultures of Nitzschia, in sea-water enriched with phosphate, are shown in Tables IV and V. It is seen that ammonium-nitrogen replaces nitrate-nitrogen in the case of this neritic diatom, and that its addition is equally effective in promoting growth.

TABLE IV.

Oxygen in c.c. per Litre Produced during Continuous Illumination.

	After 18½ hr.	42½ hr.	90½ hr.	138½ hr.	Increase due to addition.
No addition . . .	0·37	0·85	1·06	1·2	
With addition of 60 mg. per m.^{3} of nitrate/N_2 .	0·52	1·7	2·4	2·5	1·3
60 mg. per m.^{3} of ammonium N_2 ammonium .	0·51	1·8	2·7	3·1	1·9

TABLE V.

OXYGEN, C.C. PER LITRE, PRODUCED AFTER

	7 hr. illumination 24½ hr. darkness 21 hr. illumination	54 hr. illumination 24½ hr. darkness	93 hr. illumination 24½ hr. darkness	Increase due to addition.
No addition . . .	0·7	2·8	4·0	
60 mg. per cubic metre nitrate–N_2 added .	1·08	4·0	5·15	1·15
Ditto	1·16	4·2	5·45	1·45
60 mg. per cubic metre of ammonium N_2 added	1·08	4·0	5·23	1·23

The increase in oxygen production brought about by this addition of 60 mg. per cubic metre of nitrate-nitrogen in these two experiments was 1·3, 1·15, 1·45 c.c. per litre, the mean being equivalent to the fixation of 0·7 gm. carbon per cubic metre. The ratio of nitrogen added to carbon fixed is therefore $\frac{1}{12}$.

Analyses by Raben of plankton consisting mainly of mixed diatoms gave a ratio of nitrogen to carbon amounting to roughly $\frac{1}{5}$. As with phosphate, the addition of a small quantity of available nitrate gives rise to an unexpectedly large increase in the amount of carbon fixed.

During the vernal outburst of diatoms in the sea about two and a half times more nitrate-nitrogen than phosphate (as P_2O_5) is used up. Yet in these experiments the addition of roughly ten times more nitrate-nitrogen than phosphate is required to bring about the same increase in fixed carbon.

Under the conditions of these two experiments the addition of ammonium brought about a similar increase in growth to that brought about by the addition of nitrate.

THE EFFECT OF ARSENATE UPON THE GROWTH OF *Nitzschia closterium*.

The quantity of phosphate in sea-water as estimated by the Atkins-Denigès method includes any arsenate which may be present. After vigorous growth of phytoplankton in the summer, the phosphate content of the water, estimated by this method, may fall to less than 1 or 2 mg. P_2O_5 per cubic metre. Hence there is either practically no arsenic present as arsenate or the phytoplankton utilises it. With regard to the latter possibility, it is known that some plants can utilise arsenate partially in lieu of phosphate. In a single preliminary experiment, made in 1930

in daylight with rather crude apparatus, increased growth was observed due to the addition of sodium arsenate amounting to 20 mg. As_2O_5 per cubic metre. However, in six instances, when between 20 and 40 mg. As_2O_5 per cubic metre were added and the experiments carried out in the apparatus here described, the rate of photosynthesis was reduced.

Effect of Hydrogen Ion Concentration.

An experiment was made with a vigorous dense growth of Nitzschia in filtered sea-water which had been enriched with phosphate and nitrate. The pH was reduced to varying degrees by blowing alveolar air through the culture. The oxygen production during illumination is shown in the following table.

TABLE VI.

Approximate pH measured colorimetrically. Initial.	Final.	c.c. oxygen produced per litre during 3 hr. 45 min. illumination.
8·4	8·45	0·48
8·1	8·1	0·50
7·9	7·9	0·57
7·45	7·4	0·55
less than 7·0		0·55

This experiment indicates that slight variations in pH in the tubes of culture are not likely to have invalidated any of the foregoing experiments.

The Effect of Excess Base.

In many of the following experiments rich cultures were produced in small flasks where, from the nature of the experiment, the rate of growth may have been gradually slowed owing to excessive rise in pH. Since the addition of any base would hinder the rise in pH by increasing the buffering, it was desirable to find out if the addition of any substances used in the experiment had acted by altering the " excess base " in the sea-water and so affecting its buffering capacity. With this end in view an experiment was made in which a solution of sodium bicarbonate saturated with carbon dioxide was added to flasks of enriched sea-water inseminated with Nitzschia. This increased the excess base to ·0036, ·0046, and ·0086 Normal. No significant effect was noticeable upon the growth of Nitzschia.

The Effect of Iron.

Filtered open sea-water was enriched with phosphate and nitrate and inseminated with Nitzschia. Varying quantities of ferric ammonium citrate were added to flasks of this. After two days in a north window the

effect of added iron was noticeable. On the ninth day the cells were counted by means of a Hæmocytometer with the following result :

Iron added. Mg Fe per c.m.	Number of cells per cubic mm.
none	5,340
8	7,070
80	9,540
1,600	13,870

It was realised from other experiments proceeding simultaneously with this one that the full effect of added iron was probably suppressed owing to lack of silicate. The cultures are thick and the diatoms require more than is initially present in the water (200–400 mg. SiO_2 per m.^{3}) and is being continually dissolved from the glass of the flasks.

A similar experiment was made in which the water was enriched with silicate as well as with phosphate and nitrate, and three series of flasks set up with additions of ferric ammonium citrate. After fifteen days in a window counts were made in one series with the following result :

Iron added. MgFe per c.m.	Number of cells per cubic mm.
none	7,370
1 mg.	16,360
3 mg.	16,540
5 mg.	28,000

From inspection as growth was proceeding in the three series of flasks it was clear that the rate of growth was about doubled in each case by the addition of 1 mg. Fe per cubic metre.

An experiment was made with a vigorous thick culture, in water enriched with phosphate nitrate and silicate, to see if the addition of iron caused division of the cells or formation of colouring matter when kept in the dark, but no evidence of this was found.

A number of experiments were made with the object of indicating in what form iron is available and effective. Iron ammonium citrate is slowly hydrolysed in sea-water, less rapidly if sodium citrate is also added, when it remains longer in solution as an " iron-citrate " complex. The addition of sodium citrate did not make a culture with iron ammonium citrate grow any faster, which indicates that the " iron citrate " complex itself is not available to the plants—a conclusion also arrived at by Hopkins (**13**). With regard to other iron compounds an experiment was made in which 800 mg. Fe per cubic metre was added in the form of ferric ammonium citrate (slowly hydrolysed), ferric alum, ferrous sulphate (both rapidly hydrolysed in sea-water) and dialysed iron, the colloidal

hydroxide formed by hydrolysis. The citrate was most immediate in its action, but after twelve days in a window the iron alum and ferrous sulphate had brought about an increased growth similar to that due to the addition of citrate. Dialysed iron in this and other experiments was found to have no effect or only very little. Gran had found that hæmoglobin was without effect and the writer that hæmatin likewise did not promote growth. A small piece of steel was found to improve the growth in a culture, as it rusted. It would seem that these additions act through the iron-ions which they give off and which escape hydrolysis to the unavailable hydroxide.

The writer was unable to detect any ferric or ferrous ions in sea-water until it had been both made acid and subjected to vigorous oxidation, as by boiling with bromine water. It would seem that it mostly occurs as organic compounds (**14**) in solution and ferric hydroxide in suspension or colloidal solution (**4**), with only a small moiety in a form available to plants.

These experiments as a whole indicate that iron in an available form does not exist in sea-water in sufficient quantity for the most rapid growth of Nitzschia. There is sufficient, however, for a heavy final production. There is no evidence whether this is present *ab initio* or is formed from " unavailable iron " as the " available " is used up.

We have found that the addition of 1 mg. Fe per cubic metre in the form of citrate doubles the rate of growth of Nitzschia. Probably much of this addition exists in the form of iron-citrate complex and the hydroxide formed from it by hydrolysis, neither of which appear to be " available." It does not seem unreasonable to conclude from this that sea-water contains less than 1 mg. per cubic metre of iron in an available form. Analysis shows that the concentration of iron-ions is less than this amount. Hopkins considers that iron-ions are the only form in which iron is available, basing his conclusion on experiments with the freshwater alga Chlorella, which however requires much more iron for growth than do marine diatoms. He estimates that Chlorella requires some 82 mg. iron-ions per cubic metre in the culture solution to give satisfactory growth.

The Effect of Added Silicate.

The filtered sea-water used in these experiments contained silicate in solution to the extent of 200–400 mg. SiO_2 per m.^{3}. A further supply of silicate arose from the glass vessels. Sea-water is known to dissolve silicate from glass, and after long use the flasks are seen to be corroded.

In a number of cases it was noticed that with a thick culture in glass flasks—2,000 to 5,000 cells per cubic mm.—increased growth was obtained if the water had been enriched with sodium silicate (2,000 mg. SiO_2 per

cubic metre) in addition to phosphate and nitrate. The cells were rather larger and more regular in shape.

The solution of silicate from the glass was demonstrated in an experiment where sea-water inseminated with Nitzschia and enriched with phosphate and nitrate was kept in a north window in glass bowls. Two of the bowls were coated with paraffin wax inside while two were coated outside in order that the illumination in all four should be similar. During the first days there was no noticeable difference, but in seven days the growth had become significantly greater in the bowls which were waxed outside and where the water was free to dissolve silicate from the glass.

The Effect of a Previous Growth of Nitzschia in the Culture Medium.

A rich culture of Nitzschia was prepared in filtered sea-water enriched with phosphate and nitrate. This culture, several weeks old, was filtered through a membrane filter and the filtrate saturated with air. As control a sample of sea-water was filtered in the same way. Both were enriched with excess of phosphate and nitrate, then seeded with the same quantity of Nitzschia, and kept in a window. Growth was significantly greater in the water filtered from the previous growth of Nitzschia.

The experiment was repeated except that a Nitzschia culture was filtered as soon as a thick vigorous growth had taken place. The filtrate was low in dissolved silica, containing 210 mg. SiO_2 per cubic metre, while the control contained twice this amount. In this case rather more growth was obtained in the control than in the filtrate from the vigorous culture. However, when a trace of sodium silicate was added to both, bringing the SiO_2 content up to about 2,000 mg. per cubic metre in each case, significantly greater growth was obtained in the filtrate from the culture.

These experiments indicate that Nitzschia does not secrete substances into the surrounding water which are inimicable to the further growth of the same species, but rather that the cells secrete substances which accelerate growth.

The Effect of Soil Extract upon Growth.

The influence of land drainage running into the sea upon the growth of diatoms has been noticed since many years. It used to be attributed to the supply of phosphates and nitrogen salts carried down from the soil, but the more recent analyses show that these often amount to little and that their effect would not extend far seaward. Gran suggests that land drainage acts largely by reason of the iron salts and colloidal iron which it brings into the sea, possibly also manganese. Atkins showed that it

provides silicate, lack of which may hinder diatom growth, perhaps even preventing it in the open oceans of low latitude far from land, where diatoms give way to coccolithophores.

It has long been known that the growth of diatoms and other plants, moulds and other organisms in culture solution is accelerated by the addition of a little vegetable or soil extract (Miquel, Allen, Pasteur, Wildiers, Schreiber, Nielsen and Hartelius and others). It appears that some organic substance or group of substances, occurring rather widely in the breakdown products of living organisms, acts as a growth-promoting factor.

The foregoing experiments show that the silicate in solution in a soil extract may often increase growth, even in cultures in glass vessels from which silicate is continuously though slowly given off. They show that diatom growth will be accelerated if the addition of soil extract to sea-water increases the available iron. No experimental evidence has actually shown that it adds to the available iron, although this seems probable.

It is yet an open question what constituents of land drainage other than silicates, phosphates, nitrogen salts and perhaps iron influence diatom growth in the sea. The following experiments were made with the aim of throwing more light upon this.

Soil extract was prepared by boiling garden soil with water, filtering and bringing this filtrate to boiling point at intervals of two days or more to destroy bacteria.

Filtered sea-water, heavily enriched with phosphate and nitrate, about 1,000 mg. P_2O_5 and 3,000 mg. nitrate-nitrogen per cubic metre, was seeded with Nitzschia and 60 c.c. portions poured into five small flasks. Addition of soil extract was made to four of these. They were kept in a north window at room temperature for nine days, when the number of cells was great enough to make fairly accurate counting possible.

TABLE VII.

NUMBER OF CELLS PER CUBIC MILLIMETRE.

	After 9 days.	
No. addition . . .	860	Increase due to addition
0·05 c.c. soil extract . .	1,030	170
0·10 c.c. ,, ,, . .	1,230	370
0·5 c.c. ,, ,, . .	1,380	520
2·0 c.c. ,, ,, . .	1,770	910

The increased growth was very apparent on inspection only, the colour of the culture being browner and the turbidity due to the tiny cells more marked. Later, as growth proceeds, the differences between the flasks

became less noticeable—the cells tend to clump together and to adhere to the bottom and sides of the flasks, making accurate counting impossible.

Experiments were then made to investigate the effect of soil extract on the rate of growth and of respiration of an actively growing culture of Nitzschia in enriched sea-water. Table VIII shows the effect of 3 hours illumination, followed by 18 hours darkness, followed by 4 hours illumination and then 18½ hours darkness.

TABLE VIII.

OXYGEN PRODUCTION +OR UTILISATION —IN C.C. PER LITRE DURING PERIODS OF

	3 hr. illumination.	18 hr. darkness.	4 hr. illumination.	18½ hr. darkness.
No addition . . .	+1·14	−0·48	+2·4	−0·68
No addition . . .	+1·14	−0·48	+2·4	0·60
With 0·5 c.c. soil extract .	+1·14	−0·53	+2·9	0·60
2·0 c.c. ,, ,, .	+1·16	−0·65	+3·3	0·68
5·0 c.c. ,, ,, .	+1·14	−0·75	+3·3	0·78

It is seen that soil extract did not affect the rate of photosynthesis during the first 3 hours illumination—it is not immediate in its effect. The respiration rate after this short period of illumination was however increased. The rate of photosynthesis was increased during the ensuing period of 4 hours illumination but the rate of respiration during the ensuing period of darkness was not materially affected.

The increase in respiration after the first short period of illumination is peculiar. The values are significant and regular. In order to see if it was due to the oxidation of the soil extract, the experiment shown in Table IX was made. Two flasks were charged with a rich growth of Nitzschia in enriched sea-water and two with filtered sea-water without diatoms. To one of each soil extract was added. After 48 hours shaking in darkness at 16° C. the lights were turned on to make sure that the soil extract was effective in promoting photosynthesis.

TABLE IX.

OXYGEN UTILISED—, IN C.C. PER LITRE

	After 24 hr. darkness.	After 48 hr. darkness.	Oxygen produced during subsequent period of 6 hr. illumination.
No addition	−1·25	−2·68	+2·2
With 5 c.c. soil extract . .	−1·31	−2·76	+3·3
Filtered sea-water only . .	−0·10	− ·17	
Ditto, with 5 c.c. soil extract .	−0·15	− ·32	

Slow oxidation of dissolved organic matter in the filtered sea-water is demonstrated, greater where soil extract had been added. Taking this into account the values show that the addition of soil extract had no appreciable effect upon the rate of respiration, although the added soil extract was very effective in promoting photosynthesis during subsequent illumination.

The next step was to find whether addition of soil extract caused division of the cells or formation of colouring matter in darkness. Filtered sea-water was enriched with phosphate and nitrate, inseminated, and after a vigorous growth of Nitzschia had developed divided into two portions. To one 10% of filtered sea-water was added, to the other 10% of an extract of soil made with sea-water. They were then kept in the dark for 48 hours and the cells counted by means of a hæmocytometer. This experiment was repeated three times with the following results :

NUMBER OF CELLS PER CUBIC MM.

	With soil extract.	Without.	Increase.
Experiment I . .	1,540	1,320	16%
,, II . .	5,630	4,840	16
,, III . .	1,630	1,490	9

There was no noticeable increase in colouring matter due to the addition of soil extract.

A series of experiments indicated that soil extract did not act only through the silicate and iron which it contains. Filtered sea-water was enriched with phosphate, nitrate, silicate and iron (80 mg. Fe per cubic metre in the form of ferric ammonium citrate), and inseminated with Nitzschia. Growth was rapid and the final production great, yet the addition of soil extract caused a material increase in the rate of growth. There is reason to suppose that this increase was not due to the extra silicate and iron added in the soil extract, since there was already a large excess of silicate and a heavy enrichment with iron in the control.

That iron does not play the leading rôle is further suggested by the observation that an alkaline extract of soil is more effective than an acid one, both being neutralised previous to use.

There is indication that the growth-promoting factor is of organic nature, possibly a group of organic substances, often present in the break-down products of animal or vegetable matter. Thus evaporation and ashing of soil extract destroys its growth-promoting properties. The addition of a very small quantity of yeast or Fucus or Nitzschia extract or of a very dilute solution of ovolecithin promoted growth in a culture rich in phosphate, nitrate, silicate and iron.

The Effect of Ice Water.

A suggestion has been made by Barnes (**15**) that the production of diatoms may be influenced by trihydrone molecules in the water from recently melted ice or snow. He found that Spirogyra grew more rapidly in water which had been previously frozen than in water which had recently condensed from the gaseous state, and even than in water which had long been in the liquid state. In this preliminary communication details of the control of other factors, such as the supply of nutrient salts, are not given.

It is easy to experiment with Nitzschia in such a way that the only variables are the possibly different nature of the water molecules. Filtered sea-water was enriched with phosphate, nitrate, iron citrate, and sodium silicate and inseminated with the diatom. Fifty c.c. portions of this were filled into small flasks, to half of which were added 10 c.c. of recently distilled water and to the other half 10 c.c. of the same water which had been frozen and allowed to melt. The flasks were then kept in a north window at room temperature, which varied around 20° C. Between each pair of flasks the only difference was that recently distilled water had been added in the one case, and water from melted ice in the other.

The experiment was repeated several times and, except in the case of one pair of flasks, a greater growth could be distinguished after several days where ice water had been added. Counts were made of the number of diatoms in one pair of flasks after five days' growth with the following result:

With recently distilled water, 4,310 cells per cubic mm.
With recently distilled water previously frozen and melted, 5,430 cells per cubic mm.

In this experiment, with two pairs of flasks, the difference was very clearly distinguishable, equally so in each pair. In other experiments the difference was not always so marked although distinguishable.

The question was attacked in another manner. Filtered sea-water similarly enriched was filled into two flasks. One was partially frozen and allowed to attain room temperature. Then both flasks were inseminated with the same quantity of a thin Nitzschia culture and kept in a north window. In each of the experiments the growth was perceptibly greater in the water which had been partially frozen. It is possible that a minute trace of some constituent separated out from solution when the latter concentrated during the partial freezing, so these experiments do not form such good indication of the possible physiological action of trihydrone as the former series appear to do.

These experiments were all made at room temperature during the

summer months under conditions of good illumination, the daylight lasting some 15 hours out of the 24.

A similar series of experiments was made in November, also at room temperature, the illumination being weaker and lasting only some 9 hours out of the 24. No difference was detected between the flasks with added ice water and the controls although good growth had taken place in a week.

I am indebted to Dr. E. J. Allen, F.R.S., and to my colleagues, particularly Dr. W. R. G. Atkins, F.R.S., Mr. F. S. Russell, and Mr. G. M. Spooner, for many suggestions and for the help which they have given me during the course of this research.

SUMMARY.

The neritic diatom *Nitzschia closterium* was grown at constant temperature under artificial illumination in filtered sea-water to which various additions had been made. The oxygen production or utilisation was measured by observing the change in volume of air with which the cultures were continuously shaken.

The addition of phosphate to a culture poor in this nutrient salt increased the rate of both photosynthesis and respiration.

If kept in darkness after the addition of phosphate, an increase in number of cells and of yellow-green pigments resulted.

The addition of 1 part P_2O_5 gave rise to an increased carbon fixation of 107 to 120 parts of carbon.

The addition of nitrate and ammonium increased the rate of photosynthesis.

The addition of 1 part nitrate-nitrogen gave rise to an increased carbon fixation of 12 parts of carbon.

The addition to sea-water, enriched with phosphate, nitrate, and silicate, of 1 mg. Fe per cubic metre in the form of citrate doubled the rate of growth.

The addition of silicate increased the production of Nitzschia in glass vessels from which the culture solution only slowly dissolves silica.

Experiments bearing on the effect of a previous growth of Nitzschia in the culture solution are described.

The addition of a sterilised extract of garden soil increased the rate of photosynthesis. It also caused multiplication of cells in the dark. Evidence is given suggesting that this may be due to the provision of silica and possibly iron in a form readily assimilated by diatoms, and also to a " growth-promoting factor."

Some evidence is given that the growth of Nitzschia proceeds faster,

under certain conditions, in water part of which had recently been in the form of ice.

The conclusions are tentative in that they are based on a limited number of observations of one species of diatom under a narrow range of experimental conditions; moreover, it is not improbable that this species may behave differently under different conditions, as of light, temperature, age and volume of culture. The aim of this preliminary investigation was to suggest new lines of enquiry and supplement current research concerning the productivity of the sea, rather than to study the effect under a wide range of conditions of any one factor upon this diatom.

REFERENCES.

1. Ruud, J. T. Nitrates and Phosphates in the Southern Seas. Journal du Conseil, Vol. 5.
2. Hart, T. J. Paper read at London meeting of the Challenger Society, Jan. 1932.
3. Gran, H. On the Conditions for the Production of Plankton in the Sea. Rapp. et Proc. Verb., Vol. 75, pp. 37–46, Copenhagen, 1931.

   —— The Spring Growth of Plankton at Möre. Skr. utgitt av det Norske Videnskaps-Akademi i Oslo. I Matem-Naturv., Klasse, 1930, No. 5.
4. Braarud, T., and Klem, A. Hydrographical and Chemical Investigations in the sea off Möre. Skr. uttgift av Hvalraadet ved Univ. Biologiske Lab., Oslo, No. 1, 1931.
5. Bigelow, H. B. Plankton of the offshore waters of the Gulf of Maine. Bur. Fish. Doc. No. 968, Washington, 1926.
6. Kreps, E., and Verjbinskaya, N. Seasonal Changes in the Barents Sea. Journ. du Conseil, Vol. 5, p. 329, 1930.
7. Wattenberg, H. Über die hydrographischen, chemischen und biologischen Verhaltniss zwischen Island und Grönland. Ann. Hydrog., Bd. 58, pp. 233–250, 1930.
8. Brujewicz, S. W. Hydrochemical work in the Barents Sea, 1927–30. Reports of the 1st session of the State Oceanographical Institute, No. 1, p. 35, Moscow, 1931.
9. Cooper, L. H. N. Chemical constituents of biological importance in the English Channel. Journ. Mar. Biol. Assoc., N.S., Vol. XVIII, pp. 677–728, 1933.

10. MARSHALL, S. M., and ORR, A. P. "The Relation of Plankton to some Chemical and Physical factors in the Clyde Sea Area." Journ. Mar. Biol. Assoc., N.S., Vol. XIV, pp. 837–868, 1927.

11. BRANDT, K., and RABEN, E. "Zur Kenntniss der chemischen Zusammensetzung des Planktons." Wiss. Meeresuntersuchungen, Kiel, Vol. 19, pp. 177–210, 1922.

12. COUPIN, H. Sur l'origine de la carapace siliceuse des Diatomées Compt. Rend. d. Acad. Sci., Vol. 175, pp. 1226–1229, Paris, 1922.

13. HOPKINS, E. Iron Concentration and Growth. Bot. Gazette, Vol. 89, p. 209, 1930.

14. HARVEY, H. W. Oxidation in Sea-water. Journ. Mar. Biol. Assoc., N.S., Vol. XIII, pp. 953–969, 1925.

15. BARNES, T. C. "The Physiological Effect of Trihydrol in water." Proc. Nat. Acad. Sci., Vol. 18, p. 136, 1932.