A quantitative consideration of some factors concerned in plant growth in water

by

W. R. G. Atkins.

Marine Biological Laboratory, Plymouth.

PART II. SOME CHEMICAL FACTORS[1])

Carbon dioxide assimilation and the alkalinity of sea water.

The energy supplied by the light enables the plankton and the fixed algae to carry on photosynthesis. They derive their carbon dioxide from that in solution in the water, which is continuously replenished by the hydrolysis of bicarbonates, the water becoming progressively more alkaline. Rise of temperature, which lessens the solubility of carbon dioxide has the same effect and necessitates the introduction
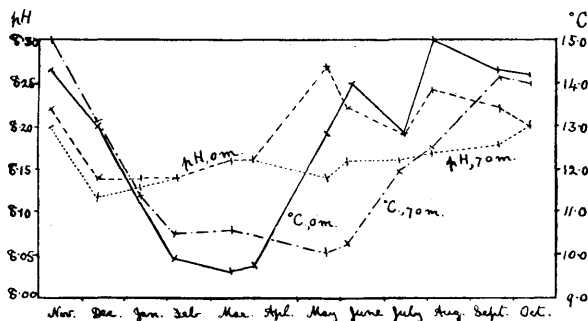

Fig. 6. Seasonal changes at Station E 1, surface and bottom, Nov. 1921 to Oct. 1922 for temperature and pH value.

of a correction before the observed seasonal change in alkalinity can be put down as due to photosynthesis. Fig. 6 shows the seasonal changes in the pH value and temperature of the sea at Station E 1, it may be

[1]) Part I appeared in the Journal, Vol. I, No. 2, pp. 99—126.

14*

noticed that the vernal rise in alkalinity reaches its maximum long before the temperature maximum is reached. Bad weather brought about a lowering of the alkalinity, partly due to mixing with deeper water. Fig. 7 illustrates this, showing the straightening out of the pH-depth curve in July. It is seen that in general the alteration in alkalinity is a phenomenon of the upper twenty metres, which is gradually extended to the deeper water by mixing. This conclusion is supported by the evidence of the temperature changes.

It should be mentioned that the symbol pH denotes the logarithm of the reciprocal of the hydrogen ion concentration expressed in grams
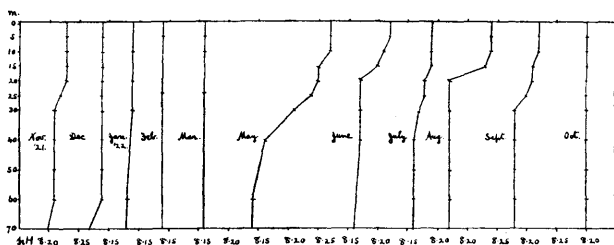

Fig. 7. Variation of pH value with depth at Station E 1, Nov. 1921 to Oct. 1922.

per litre, which for hydrogen is the same as expressing it in gram equivalents. Since the product of the hydrogen and hydroxyl ion concentrations is a constant, we may state alkalinity in terms either of hydrogen ions — which decrease — or of hydroxyl ions, which increase, as the alkalinity increases. The symbols C_H and C_{OH} are used to denote the actual concentrations of the ions in gram-equivalents. The data given for C_{OH} in Fig. 8 are in milligram-equivalents, which, as the equivalent weight of the hydroxyl ion is 17, afford actual weights when multiplied by this number. In plotting Fig. 8, in order to bring out the relation between duration of the day, sunshine and alkalinity, use has been made of C_{OH} as well as pH values, for the latter being on a logarithmic scale, tend to render the magnitude of the concentration changes less readily understood. Since, however, natural waters range from about pH 4.6 in bogs to pH 9.6 in calcareous ponds subject to intense photosynthetic activity, it is convenient to use the logarithmic scale, especially for graphic representation, as the changes amount to one hundred thousand-fold. Fig. 8 also shows the reduction in carbon dioxide pressure corresponding to such alterations in alkalinity as are plotted above, the former being obtained from scales constructed by McCLENDON (1917) for the

equilibrium pressures of various bicarbonate solutions. His buffer mixtures were also used in carrying out the pH determinations.

Inspection of Fig. 8 renders it evident that there is a causal connection
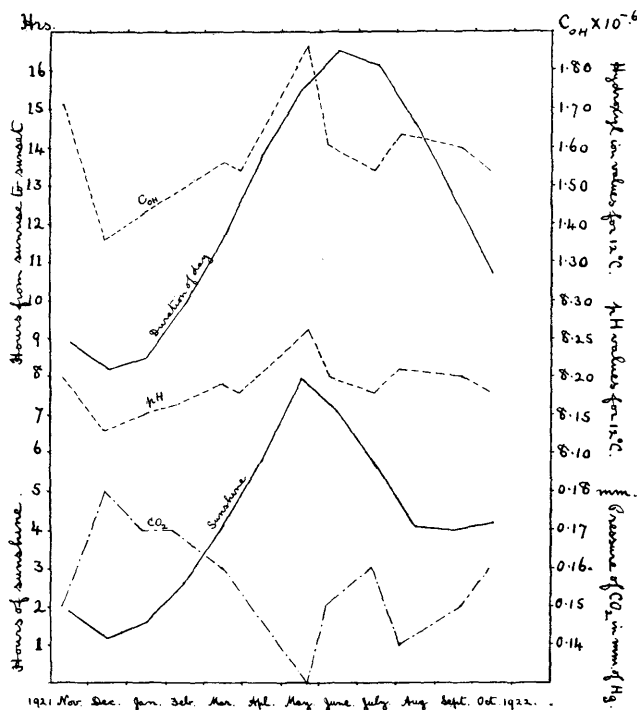

Fig. 8. Seasonal changes at Station E1, surface. The C_{OH} concentrations and the CO_2 pressures have been deduced from the pH measurements. The data for sunshine and duration of the day are taken from the records for England S.W. area and have been plotted as values for the 15th day of each month.

between the increase in alkalinity and the onset of long days with abundance of sunshine. The dip in the alkalinity curve for July, noticed already in Figs. 6 and 7 did not occur in 1923, for which year the observed surface maximum was slightly lower than in 1922 and was recorded for early July, instead of the latter part of May.
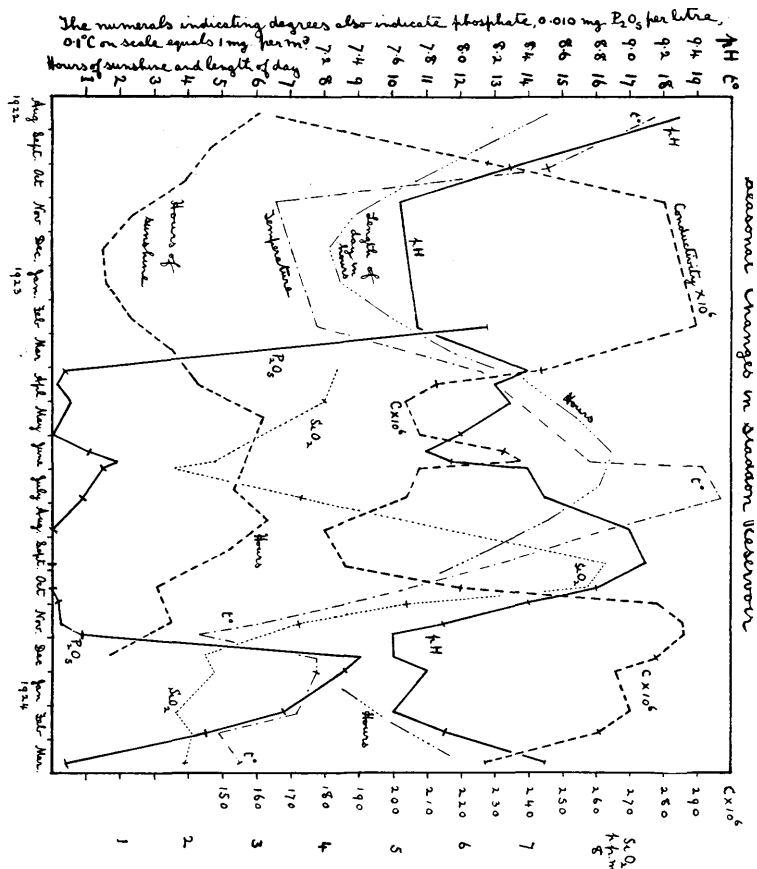
Fig. 9. Seasonal changes in fresh water. Note the large variations in temperature, pH value and electrical conductivity, also in phosphate and silicate. The latter will be considered later.

In Fig. 9 is shown the seasonal change in pH taking place in a small fresh water reservoir. Instead of a range of from about pH 8.12—8.28 as in the sea at E 1, the fresh water — being richer in plant life and being less highly buffered against change on account of its smaller content

of magnesium bicarbonate — varied from about pH 7.6 in winter to 9.3 in summer, with a secondary maximum in spring. Fig. 9 also records the seasonal changes in the electrical conductivity of the water. These are large and vary inversely as the pH values, because apparently the conductivity is mainly due to bicarbonates. As photosynthesis abstracts carbon dioxide the latter are progressively converted into the less soluble carbonates and precipitated. Other salts are also used up when plant growth is active, hence the conductivity falls. In sea water the smaller pH range and the vastly larger quantities of chlorides and sulphates render the conductivity change inconspicuous.

It has been noted that in the sea water the pH range is relatively small. This, however, is because it is sparsely populated. In a jar *Ulva latissima* was observed to bring the alkalinity up to pH 9.7 when in direct sunlight and a pure culture of the diatom *Nitzschia closterium* W.SM., in a good north light became progressively more alkaline, so that starting at pH 8.2 when the culture was young it eventually reached pH 9.4.

Up to this, alteration in pH has only been reviewed in so far as it concerns the relatively shallow water of the English Channel. PALITZSCH (1911, 1912) obtained the results given in table 8, which deal with deep water; those for the Faroe Is. were determined in May and June, and the Mediterranean values between June and September. PALITZSCH also looked for a relation between increase in alkalinity and in oxygen content of the water. In general a region of high pH value is also high in oxygen, as a natural result of photosynthetic activity. Low pH values are found in regions where oxygen is reduced in amount, or even absent, as in the Black Sea and the lower strata of certain fresh water lakes in which a well marked epilimnion has long persisted. Oxygen is usually present in the upper 100 metres of the open sea to an extent not far from saturation and as its lack never hinders the growth of the phytoplankton it will not be further considered here. GAARDER (1917) and HELLAND-HANSEN (1914) studied the relation between the pH value and oxygen saturation the latter working notably in the open Atlantic. GAARDER's figures for C_{OH} and O_2 in Inderopollen bring out the relation clearly.

Inspection of the table makes it clear that the deeper water, far removed from photosynthetic action and subject to relatively little vertical mixing, is a region in which carbon dioxide accumulates in excess of the partial pressure at which it is in equilibrium with its own content in the air. The accumulation appears to be due to the decomposition of animal and plant remains which also enrich the deeper waters

Table 8.

Depth in metres	N. Sea, E. of Faroe Is. p H	Atlantic W. of Portugal p H	Mediterranean between Sardinia and Italy p H	Black Sea
0	8.13	8.22	8.23	8.34
100	8.09	8.13	8.21	7.86
400	8.03	8.04	8.19	7.53[1]
1,000	7.98[2]	8.01	8.14	7.26
2,000	—	7.95	8.09	—
3,200	—	—	8.07	—

with phosphates and nitrates as shown in Table 9 (ATKINS and HARVEY 1925). The silicate content too increases with depth. The analyses were made upon water, obtained between Lisbon and the Canary Is. by Dr. KEMP in the R. R. S. "Discovery", which was examined in Plymouth slightly over a fortnight later. The bright sunshine and high water temperature of this region combine to give remarkably high pH values down to 75 m. The agreement of the 1000 and 2000 m. values in this series and in PALITZSCH's off Portugal is striking, the readings being pH 8.03 and 7.94 for ours and pH 8.01 and 7.95 for his samples.

Table 9. Analyses of water from 37°44′ N., 13°21′ W., taken Oct. 12th, 1925, save the last taken Oct. 16th at 29°59′ N., 15°03′ W.

Depth in metres	t° C.	pH	In mg. per m³		
			Phosphate as P_2O_5	Nitrate as N_2	Silicate as SiO_2
0	21.10	8.35	0	15; 11	
10	21.10	—	—	7	
20	21.00	—	—	6	
30	21.00	—	—	—	220
40	21.00	—	—	16	
50	20.01	8.35	0	ca. 6	
75	17.31	8.31	5	6	
100	15.10	8.18	8	55	220
150	15.06	8.16	10	65	
200	13.86	8.11	22	100	
300	12.25	8.12	44	178; 158	250
500	10.94	8.00	50	200	280
1,000	9.55	8.03	74	264; 262; 274	450
2,000	4.81	7.94	78	ca. 265	480
3,000	3.10	7.87	88	ca. 265	1,200

[1] H_2S below 180 m.   [2] 700 m.

The foregoing examples must suffice to illustrate the magnitudes of the pH values met with in sea water. The writer has summarised previous work (1922, 1) and LEGENDRE's comprehensive monograph (1925) is also available. It must be mentioned, however, that quantitative determinations of the carbon assimilation of the phytoplankton — and so an estimate of the total crop per unit area — can be made by means of pH measurements as is illustrated by the calculation below. For this it is necessary to ascertain the change in pH produced by the addition of an acid, preferably in $N/_{100}$ concentration, in varying amounts, and to note the equivalent quantity of carbon dioxide required to produce the change. Having once constructed this curve the quantities of carbon dioxide concerned in an alteration of say pH 8.14 to 8.20 can be read off. The result can of course be obtained simply, though less accurately by titrating the sea water using phenol-phthalein as indicator and noting the difference between the winter and spring or summer values. MOORE, PRIDEAUX and HERDMAN (1915) at Port Erin found such seasonal differences in titrating the water of the Irish Sea. Similar differences in fresh water had already been recorded by BIRGE and JUDAY (1911) and by CHAMBERS (1912), but it remained for MOORE and his co-workers to base a calculation of the annual crop upon the results. They assumed that the changes observed at the surface extended to 100 m. owing to mixing and arrived at an estimate of two tons dry weight or ten tons of moist plant per acre. Since the surface alkalinity is always reduced by mixing with the deeper water this result is rather too high.

A large number of titrations were made (1922, 1) during 1921 of the seasonal variations in alkalinity at and around Station E 1 and at E 2, these when averaged afforded the value 1.0 c.c. of $N/_{100}$ H_2SO_4 per 100 c.c. of water, which corresponds to 4.4 mg. per litre of carbon dioxide or to 1.2 mg. expressed as carbon. Since a sugar such as dextrose contains 40 per cent of this element, the amount suffices to provide 3 mg. per litre of dextrose, or slightly more than that amount of starch. This is equivalent to 3 grams of dextrose per cubic metre.

Taking the depth of the English Channel around E 2 as 83.3 m., which is very approximately correct, the carbohydrate expressed as dextrose synthesised in the column of water with a base of four square metres is one kilogram. Over an area of one square kilometre this amounts to 250 metric tons.

Were photosynthesis to remain uniform and respiration in abeyance it is obvious that there would be a vast accumulation of carbohydrate in the sea. As it is the amount present is an equilibrium between the

production due to photosynthesis and the destruction by plant and animal respiration.

The conditions in nature are regulated by illumination and by the amounts of the necessary salts which are available. On considering BLACKMAN's work on limiting factors and the quantitative results obtained by Miss MATTHAEI (1905), it is seen that an increase of temperature may effect a very decided increase in the rate of photosynthesis. If, however, the light intensity is low, a point is reached beyond which a further temperature rise, even when not injurious, is unaccompanied by any rise in assimilation. The amount of light energy then available is insufficient to decompose carbon dioxide at a rate in excess of that already attained at the lower temperature. From the results obtained, when light was not the limiting factor, KANITZ (1915) has shown that the van't Hoff rule holds approximately, the ratio being 2.40 per $10°$ C. rise from $0°$—$10°$ and 2.12 from $10°$—$20°$. For respiration similar coefficients are given so it is only when light is a limiting factor that increase of temperature is unattended by increase in photosynthesis as well as in respiration, provided injurious extremes are avoided.

Since alteration in temperature affects the bicarbonate equilibrium, a rise tending to increase the alkalinity, the value 1.0 c.c. of $N/_{100} H_2SO_4$ as determined by titration is subject to a correction. It appears that the correction cannot exceed 0.3 c.c. under the conditions studied. This would reduce the estimated crop by 30 per cent. The pH range for E 1 was observed to be 0.13, or corrected for the part possibly due to the establishment of a temperature change equilibrium, the range is pH 0.10. It is doubtful, however, that equilibrium is ever reached, especially when KROGH's determinations of the rate of exchange of CO_2 between air and sea water are borne in mind. The range found, pH 0.10, corresponds to 1.0 c.c. of acid very approximately, so the figure 250 metric tons per square kilometre may stand as an approximate value for the carbohydrate photosynthesised.

The phosphate content of natural waters as a factor limiting the production of phytoplankton.

A brief mention has already been made of the amounts of phosphate, nitrate and silicate in sea-water, data concerning which have been presented in Table 9. These analyses of deep Atlantic water, made quite recently, afford information that has long been wanted and may be discussed here before the earlier work has been considered.

Inspection of the table shows clearly that all three are present in

minute amount; only in the deepest water is the most abundant —
silicate — in excess of one part per million. The analyses are expressed
in milligrams per cubic metre, which for fresh water would be in parts
per thousand million; for salt water the latter number would require
to be somewhat raised on account of its greater density. It is noticeable
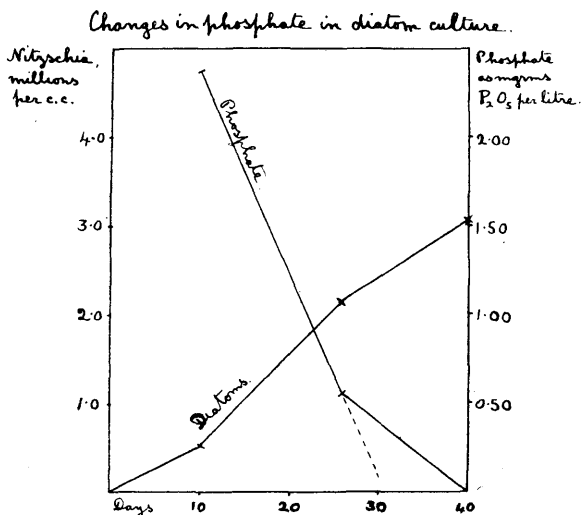moreover that all phosphate has been removed from the surface water

Fig. 10.

and but little nitrate has been left. The silicate, though much reduced,
is still present in relatively greater amount. The indication is that the
phytoplankton is responsible for the removal of these nutrient salts.
Fig. 10 illustrates the change in phosphate as the diatom *Nitzschia
closterium* multiplies in sea water enriched with MIQUEL's solution.
It was found that 1×10^9 diatoms used up 1.12 mg. of phosphate,
reckoned as P_2O_5, accordingly one gram should suffice for 9×10^{11}
Nitzschias. Data which will be presented later give 30 mg. per m³ for
the annual consumption of phosphate in the water at E 1, taking the
average of 70 m., surface to bottom. From this it follows that if all this
phosphate were abstracted by diatoms each cubic metre of sea water
would produce 26.8×10^9 diatoms. As many as 30×10^9 of another

species of diatom were found by the writer in a fresh water pond, so these large numbers, as calculated, need not seem impossible. Nothing approaching such a number of diatoms is, however, present in the sea simultaneously, and the figure for plankton organisms in summer found by ALLEN (1919) using the cultural method amounts only to 464×10^6 per m³, or 0.464×10^9, about one sixtieth of the number calculated as possible. In view, however, of the differences in the size of the various organisms and of the fact that the zooplankton, many of which are of relatively enormous mass, consumes the phytoplankton, the agreement between the estimate and the measurement is as close as could be expected.

It is of interest to consider what volume of water is stripped of its phosphate by one diatom, for since the diatom floats freely in the water it can only draw on a relatively limited water volume. Assuming that the water contains 30 mg. per m³ of phosphate, as it does early in the spring, and making use of the result of the experiment shown in Fig. 10, that one gram of phosphate, reckoned as P_2O_5 suffices for 9×10^{11} *Nitzschias*, it is found that each diatom has only to strip one twenty seventh part, viz 0.037, of a cubic millimetre of water. In other words there may be 27 diatoms per cubic millimetre; as found before the value was 26.8, merely due to a rounding off in the evaluation of the constant. At the limit of what can be detected, 1 mg. per m³, each diatom has to strip over one cubic millimetre in order to get enough phosphate, consequently the greater the dilution the slower must be the rate at which further diminution in phosphate proceeds.

The earlier analyses of the phosphate content of sea water have been reviewed by MATTHEWS (1916), RABEN (1920), and BRANDT (1920); they are all vitiated by analytical imperfections. With samples taken just outside Plymouth Breakwater MATTHEWS found a maximum of 60 mg. per m³ at the end of December, 1915, with an irregular fall to a minimum of less than 10 mg. per m³ in April and May. He attributes the seasonal variation to the removal of phosphates from solution by the fixed algae, the diatoms and *Phaeocystis*, a unicellular and primitive brown alga which occurs in these waters very abundantly in late spring. The minute quantity of phosphate in sea water may be realized when it is mentioned that, using the purest chemicals, MATTHEWS was unable to prepare artificial sea water containing less than 28.6 mg. per m³, which is only a little less than the winter maximum value at E 1.

RABEN's analyses extend from 1904—14, and include numerous determinations upon the water of the North Sea, Baltic, Barentz Sea and North Atlantic Gulf stream. These, as plotted by Brandt, show
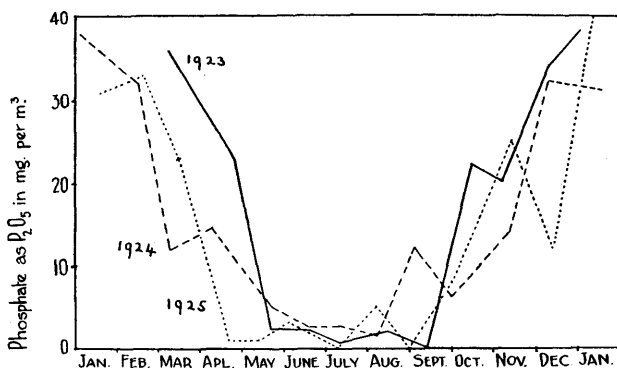
Fig. 11. Seasonal changes in phosphate concentration at Station E 1, surface.

minimal values in May and June. After a rise to a peak in September low values are again shown early in October. BRANDT's graph, like that given by MATTHEWS, refers to surface water, though RABEN also analysed water down to 800 m. in the N. Atlantic. There is usually a considerable increase from surface downwards. None of the values, however, indicate exhaustion of the water as regards phosphate, the minimum recorded figure being 51 mg. of P_2O_5 per m^3 and the maximum 221 mg., both being from North Sea Station N 7. These figures are about four times as great as those given by MATTHEWS, whose results
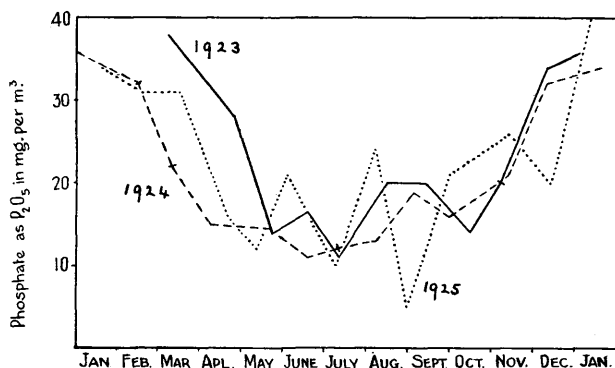

Fig. 12. Seasonal changes in phosphate concentration at Station E 1, bottom, 70 metres.

agree well with those of the Government Chemist, London, who also used POUGET and CHOUCHAK's method (1909, 1911). The analyses carried out by the writer according to DENIGÈS' method (1921), which is entirely different, are in close accord with MATTHEWS' results. Owing to an analytical error, the source of which will, it is hoped, be pointed out
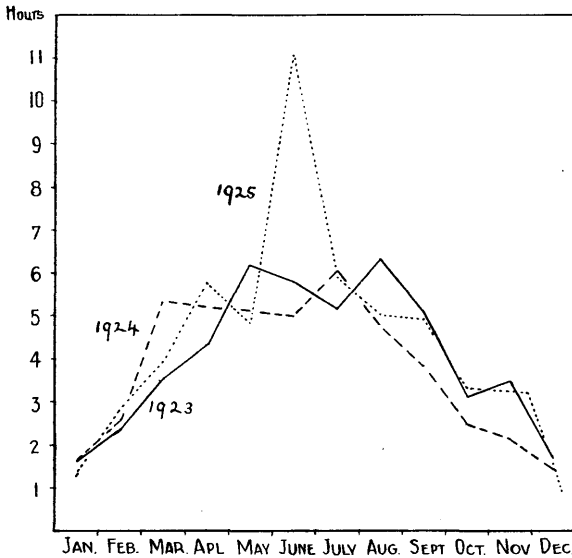

Fig. 13. Mean monthly sunshine records for England S.W. (including Wales), plotted for 15th of each month. Note the high values for spring sunshine in 1924.

elsewhere, RABEN's values are all too high and so mask the fact that phosphate is at times completely used up in surface water and accordingly becomes the factor which, under these conditions, limits the development of the phytoplankton.

A study of the seasonal changes at Station E 1 may be advanced in support of the foregoing. Figs. 11 and 12 show how the phosphate varied in the surface and bottom (70 m.) water respectively during the years 1923, 1924 and 1925. The curves are qualitatively very similar, but show notable quantitative differences. Of these the most striking are that in both figures the times, at which the vernal decrease in phosphate becomes manifest, are markedly different, 1924 being about two months

earlier than 1923, with 1925 occupying an intermediate position. Inspection of Fig. 13 gives the answer to the problem raised by these differences, for in 1924 the early spring was characterised by much sunshine, whereas in 1923 as high values were not obtained till two months later, 1925 being again in a mean position. Once the phosphate near the surface, and so in an adequately illuminated region was all used up, a further increase in illumination could obviously have no further effect there.
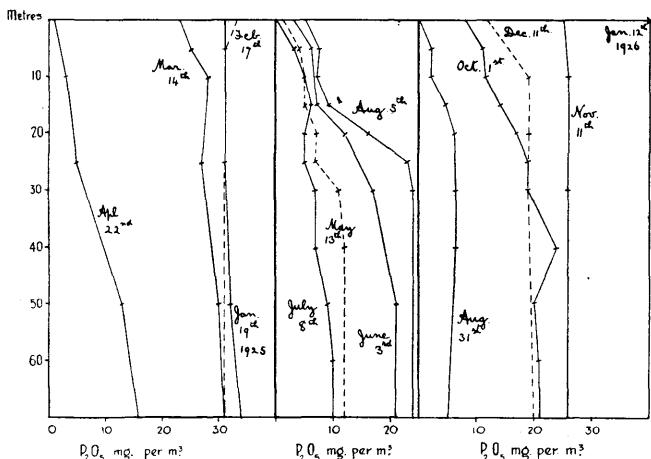

Fig. 14. Variation of phosphate concentration with depth at Station E 1 during 1925. For the sake of clearness the curves for the months have been plotted in three sections.

At greater depths an increase in intensity and in duration of illumination result in a depletion in phosphate, similar to that near the surface; this may naturally be looked for later in the year. Beyond a certain depth, however, which varies with the surface illumination and the efficiency with which it is transmitted, down-grade processes predominate and phosphate is regenerated. Hence the sharp peaks in the mid-summer portion of Fig. 12. These do not occur earlier in the year, for then the vertical circulation of the water due to the action of waves, wind and to the residual effect of surface cooling, is bringing up fresh supplies continuously. But when the warm upper layer or epithalassa is once established vertical communication is greatly diminished. A study of the temperature differences over the periods during which regeneration

of phosphate became marked shows clearly that little or no mixing of surface and bottom water could have taken place. Fig. 14 constitutes a record of the phosphate concentration at E 1 during 1925, the year
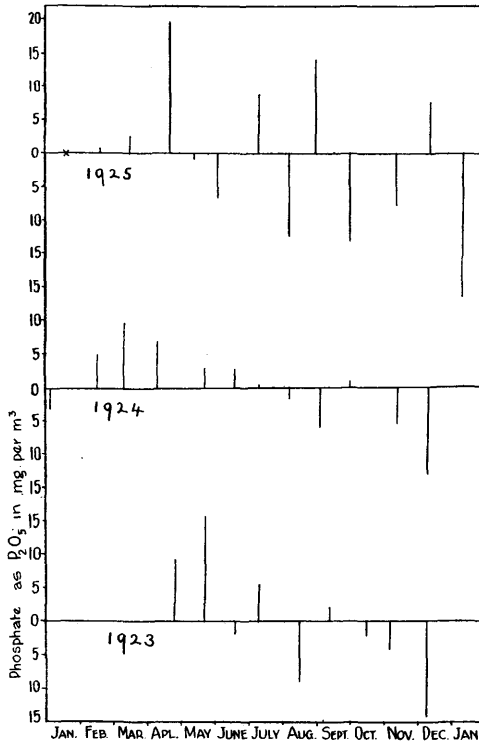

Fig. 15. The production of phytoplankton may be deduced from the uprights above the zero line; in reality these lines show the difference in the phosphate content of the water between two successive observations. Increase in phosphate, being equivalent to destruction of phytoplankton is represented below the zero line.

being divided into three periods, of four months each, for the sake of clearness. The shifting of a curve to the left denotes decrease, to the right increase. Use has been made of data of Fig. 14 to ascertain the average phosphate concentration in the water column of 70 metres. Since the

phosphate is used up in producing the phytoplankton the amount of such increase over any period may be taken as the difference between the initial and final concentrations. Such differences are shown in Fig. 15, those above the zero line representing outbursts of plant growth, those below representing regeneration of phosphate. These represent, however, the debit or credit balances, noted at intervals, of a current account. The species dominant at one period may later be replaced by another, though if consumption and regeneration balanced no indication of any such change would have been given. Fig. 15 serves, however, to help us to trace the major changes taking place in the phytoplankton. Thus considered it is seen that 1924 was characterised by an orderly sequence, with evidence of only a slight autumnal outburst; but it must be pointed out that this may have occurred in the six-weeks gap in the observations from Oct. 1st to mid November.[1]) 1923 and 1925 are similar in being characterised by notable outbursts of growth, those in July, late August and December being remarkable, especially the last, which was accompanied by the development of a *Rhizosolenia robusta* maximum, other members of the species being also present at this very unusual period of the year. In making the foregoing deductions it has been assumed that there have been no considerable water movements such as to upset the validity of the phosphate cycle. The salinity data for the period negative the suggestion of extensive water movements, save in so far as concerns the winter of 1925 for which no salinity data are as yet available.

By subtracting the consecutive values for the mean phosphate content of the water column at E 1 it is possible to arrive at an annual balance sheet, as shown in Table 10. In constructing this the maximum value given for the winter is held to include the early January result of the following year if this is greater than the December value.

It should be added that during the mid-winter period the sea is relatively poor in phytoplankton around the British Isles, hence the phytoplankton crop calculated from the phosphate changes is an approach to a full measure, though always somewhat too low.

From Table 10 it may be deduced that the phosphate cycle at E 1 was a closed system during the years studied. It is possible, however, that periods of influx of water may either increase or decrease the phosphate content at E 1. For example, from the incomplete data available

---

[1]) Owing to weather conditions and other demands on the boat it has not always been possible to make observations at regular or desirable intervals. Furthermore it is only of late that it has been borne in upon the writer how great may be the changes in phosphate concentration taking place in a relatively short time.

Table 10. Phosphate balance sheet from March 1923 to January 1926 inclusive, shown as milligrams of P_2O_5 per m^3, for the 70 m. water column at E 1.

Year	1923	1924	1925	1923—25
Total observable consumption...	33.4	29.5	53.8	—
Maximum minus minimum	29.6	28.3	26.9	—
Total observable regeneration ...	33.4	24.5	61.7	—
Gain in free phosphate, difference of successive maxima.........	— 3.0	— 5.0	+ 8.0	0.0
Gain in free phosphate, consumption minus regeneration	0.0	— 5.0	+ 7.9	+ 2.9

for the region of the Faroe-Shetland Channel it appears that the water there was considerably richer in phosphate during the summer of 1925 than it was during 1924. Quite in keeping with this was the observed increase of the phosphate content of the North Sea water in May 1925 as compared with roughly the same season in 1924. This is suggestive of the possibility of the entry of richer water, from the unilluminated depths of the Atlantic, into the North Sea via the North of Scotland. No conclusion can be based upon the data now available, which are, however, entirely favourable to this hypothesis.

Analyses made of water from the Mediterranean and between England and S. America show that the surface water of these sunny regions is almost or altogether stripped of phosphate. The deep water, as recorded in Table 9 is rich. Owing, however, to the fact that no great cooling occurs in winter, vertical mixing proceeds to lesser depths the further one moves towards the equator. Near it, however, there is evidence of some upwelling of deep water which replaces that driven polewards by the trade winds. In fact as the range of the annual temperature variation becomes reduced, so also is that of the phosphate cycle. Furthermore since dead organisms sink as they finally disintegrate and since the ocean waters are very deep it is obvious that the bottom water — or the water sufficiently deep to become really rich in phosphate — will not enter into the vertical circulation at all. Thus a lesser phytoplankton might be expected as the temperate zones are left, which is precisely what is found as may be seen from LOHMANN's (1920) results. JESPERSEN's (1924) graph for the Atlantic macroplankton also brings out clearly how much richer the coastal zones are than the open ocean where the stratification of the water is not disturbed by the effects due to proximity to land or to shallow water areas.

The richness of northern waters may accordingly be considered as due to the better vertical circulation as regards the temperate zone; also to the fact, which becomes more marked in high latitudes, that since light is the limiting factor for plant growth for a considerable portion of the year, the water accumulates a greater store of phosphate. Then when daylight returns, or lengthens its duration, a great outburst of the phytoplankton takes place.

Two further deductions may be made from a study of the phosphate cycle. It has been shown that a measure of the time and magnitude of the spring outburst of phytoplankton is given by analyses of the phosphate content of the water; this renewal of activity has been found to be dependent upon the quantity of light available rather than upon the temperature of the water. Animals, however, are regulated as regards reproduction and growth rather by temperature than by light. From this it follows that the hatching out of the young of various species which are dependent, directly or indirectly, upon the vernal outburst of the phytoplankton may take place before an adequate supply of the latter has become available and there are obviously many gradations between times of famine and of plenty. Thus this suggestion as to seasonal variations in the zooplankton, which has long been current, receives an added measure of support.

Again a knowledge of the phosphate consumption enables an estimate to be made of the minor limit of the annual phytoplankton crop. The limit is minor if based upon the figure "maximum minus minimum" in Table 10. If based on the "total observable consumption" results in this table it will still be too low, for this is only a balance figure as already explained. Taking the consumption as 30 mg. per m^3 to a depth of 70 m. at E 1 and that the phosphate content of the plankton is 0.15 per cent., calculated on the wet weight, as for the brown algae, it results that the column of water produces 1.4 kilograms of phytoplankton. If it be assumed that the carbon content of the algae, reckoned as a hexose sugar, amounts to 15 per cent. of the wet weight the calculation previously given, based on the seasonal change in alkalinity, gives an identical value 1.4 kg., when recalculated for 70 m. depth instead of 83.3 m. The exact agreement is fortuitous, but it lends support to the validity of the titration method, which is less exact than is the phosphate estimation. Thus the minimum value for the crop is 1,400 metric tons, wet weight, per annum, per square kilometre.

Fig. 9 shows, among other things, how the phosphate content of the water of a small reservoir undergoes seasonal changes so that its winter value, which is high owing to the drainage from the surface of pastures

stocked with cows, rapidly falls in spring. Similar results were obtained in a quarry pond, and the phenomenon appears to be perfectly general in all fresh waters.

The silicate content of natural waters.

In Fig. 9 is also shown the changes in the silicate content of the small reservoir. The total range is from about 2 mg. of silicate, estimated as SiO_2 per litre, up to 8 mg. — or 2000 to 8000 mg. per m^3, quantities vastly greater than the phosphate content. The sequence of the changes is hard to interpret since matters are complicated by surface drainage, but some slight portion of the decrease, and a larger proportion in another pond, appears to have been occasioned by the removal of the silicate by diatoms to form the siliceous valves

BRANDT (1920) has recorded a seasonal change in the Baltic, silicate as silica varying from 900 mg. per m^3 in February to 600 in May, followed
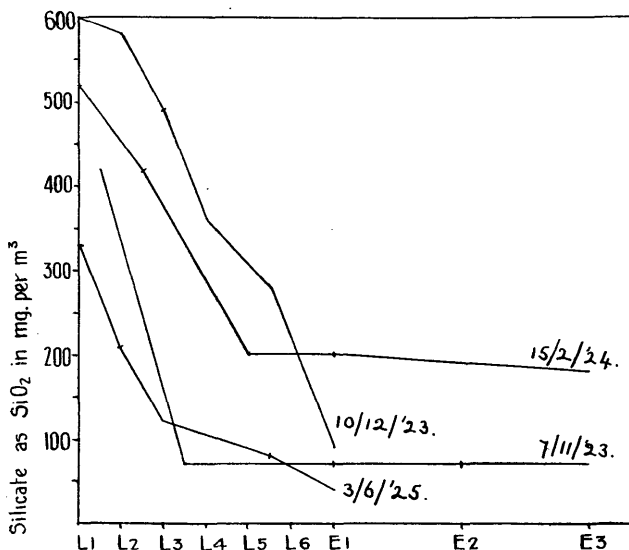

Fig. 16. Decrease of silicate from Plymouth Sound to open sea of English Channel. The L stations are at approximately equal distances apart and E 2 is midway between E 1 and E 3, which is off Ushant, but the distances are not shown to scale, that between the E stations being relatively much reduced.

by a rise in June. Bottom water in February contained 1150 mg. As the Baltic receives large accessions of fresh water and is land-locked, it is to be expected that its water should be richer in silicate than is the English Channel and the Atlantic. Table 9 shows that only at great depths in the Atlantic is water found which is as rich as is the shallow water of the Baltic. This indicates that the surface water of the Atlantic
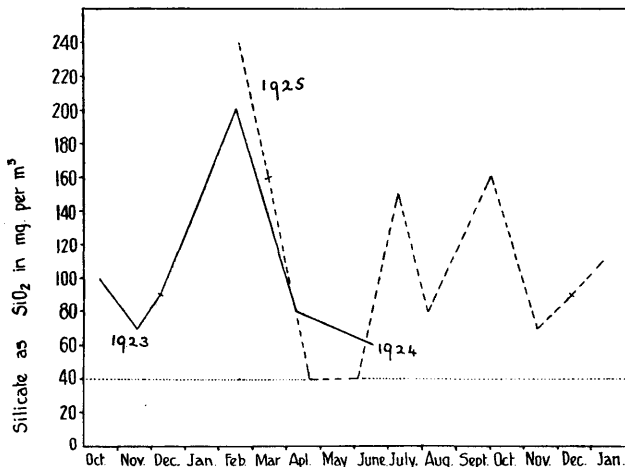

Fig. 17. Seasonal variation in silicate at Station E 1, surface. The dotted horizontal line at 40 mg. per m³ denotes the limiting amount of silica detectable. A value of 40 mg. may therefore be any lesser amount.

is poorer in silica because the latter has been depleted by the development of diatoms. It should be added that the silica values recorded in Table 9 may be somewhat high owing to the action of the alkaline sea water upon the glass. The error is negligible in freshly drawn water from the colder English Channel and is probably not serious for the figures mentioned previously; though lower values were obtained from similar Atlantic water in winter, when the solvent action would have been less, there was no indication of the silica content limiting diatom production in the open ocean.

The difference in the silicate content of Plymouth Sound and of the coastal waters as compared with the true Channel water is shown in Fig. 16 and in Fig. 17 the seasonal variations at E 1 have been plotted. Fig. 18 has been constructed for silicate precisely as Fig. 15 was for

phosphate. Over the limited period Jan. 1925 to Jan. 1926 inclusive the analyses denote a depletion of silica to the extent of 130 mg. per m³. The curve for 1925 in Fig. 17 makes it evident that it is possible that during April and May the silicate was all used up and so, equally with the phosphate, may have set a limit to the production of one class of
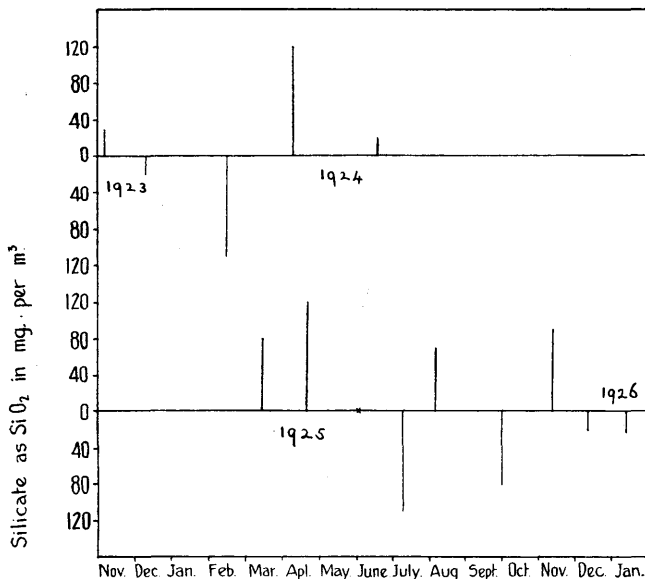
Fig. 18. Periods of production of diatoms as judged by upward lines, corresponding to mg. per m³ of silicate, as silica, used up. Downward lines denote enrichment in silicate, due to its regeneration from the decay of diatoms or from other sources.

the phytoplankton, to wit the diatoms. This is suggested because 40 mg. per m³ is the limit of silica detectable, accordingly this amount may signify any lesser quantity; a dotted line has accordingly been drawn at this level. Above this amount increments of 20 or even of 10 mg. per m³ are measurable. The limit ascertainable, 40 mg. per m³ would, however, be the winter maximum for phosphate.

The nitrogen compounds of natural waters.

The nitrogen compounds present in water have been subjected to much attention, but on account of the analytical difficulties attending

such work, more especially as regards sea-water, the number of workers who have obtained more than a few results is small.

Firstly we are in ignorance as to the source of these compounds, save in so far as they are derived from the land. Evidence pointing to the fixation of gaseous nitrogen by the higher plants has been brought forward by a number of workers, whose cultures were, however, not free from bacterial contamination. Of these WANN (1921) is noteworthy for the care he exercised and the multiplicity of his experiments. Working with pure cultures of unicellular fresh-water algae, he found that fixation only took place when the culture had been supplied with nitrate and glucose. SCHRAMM (1914) had previously experimented with a number of species and demonstrated that in the absence of added nitrogen compounds no fixation took place, accordingly WANN included a little nitrate. BRISTOL and PAGE (1923), however, showed that WANN's analytical procedure involved a loss of nitrogen at a certain stage, and the more nitrate present the greater was the loss. Consequently when the algae had used up much of the nitrate the loss was reduced and so as compared with the control a gain was indicated. Their own cultures showed no gain and were a complete vindication of SCHRAMM's conclusion in support of the views held since the early work of LAWES and GILBERT. Bacteria in the gelatinous sheathing in which algae are encased may perhaps be responsible for some such fixation.

JUDAY (1925) and his co-workers have recently made a detailed qualitative and quantitative investigation of the nitrogen compounds in the waters of Wisconsin lakes. A few analyses by DREW (1914) of the water of ponds and their vernal changes are also extant.

The Wisconsin lakes were proved to contain the following quantities as shown in Table 11.

For these analyses large volumes of water were evaporated down to small bulk, a procedure which is not possible in the case of sea-water. It will be noticed that the intermediate break-down products of living matter predominate over the final stage, nitrate. Furthermore even in summer the nitrate nitrogen remaining in the lake water is not very greatly less than the plankton nitrogen. There is a complete absence of any evidence that in the water of these lakes a deficiency of nitrogen acts as a factor limiting the plankton. The total soluble nitrogen, organic nitrogen, inorganic nitrogen and plankton nitrogen were determined for the surface and bottom water of Lake Mendota from June 1922 till May 1924. Throughout this period the surface plankton nitrogen was far less than the organic nitrogen — about one fourth — and save in June and July 1922 it was less than the inorganic. It must be remembered

Table 11. Forms of nitrogen in Lake Mendota water, in mg. per m³.

No.		Surface June 18, 1924		Bottom[1]) June 25, 1924	
I	Plankton[2])........................	—	92.4	—	44.9
II	Soluble nitrogen..................	—	515.6	—	766.9
	1. Free saline ammonia nitrogen..	96.0	—	280.0	—
	2. Residual saline ammonia nitrogen	16.0	—	20.0	—
	3. Nitrite nitrogen	10.0	—	17.0	—
	4. Nitrate nitrogen	69.4	—	92.6	—
	5. Free amino nitrogen	54.0	—	81.0	—
	6. Peptide nitrogen	135.0	—	140.4	—
	7. $1/2$ of tryptophane nitrogen....	5.3	—	7.0	—
	8. $2/3$ of histidine nitrogen	3.8	—	6.8	—
	9. $3/4$ of arginine nitrogen	31.1	—	34.7	—
	10. Amide nitrogen	12.4	—	19.3	—
	11. Purine nitrogen	8.4	—	9.5	—
	12. Amine nitrogen	14.2	—	16.0	—
III	Forms of soluble nitrogen determined, total	—	455.6	—	724.3
IV	Undetermined nitrogen............	—	60.0	—	41.9

though that the plankton as thus obtained from water pumped up will have included only the smaller members of the zooplankton. It was found that all forms of nitrogen (save that in plankton) reached a maximum in the winter and a minimum during summer.

The methods applicable sea water have been worked out by RABEN (see Brandt 1920) and similar or other methods have been studied by WITTING (1914), BUCH (1915, 1923), GAD-ANDRESEN (1923) and ALSTERBERG (1924). The elaborate researches of BRANDT, RABEN and their co-workers have given us almost the whole of our knowledge of the saline and albumenoid ammonia of sea water and its seasonal changes, accompanied by analyses of nitrate and nitrite.

The estimation of nitrite in sea water may be performed colorimetrically by means of the GRIESS-ILOSVAY reagent as pointed out by BUCH (1923) and HARVEY (1926). ORR (1926) gives analyses made by this method upon water in the Clyde Estuary and adjacent channels. Nitrite

[1]) The bottom sample was pumped up from 20 metres below the surface where the lake is about 23 metres deep.

[2]) As determined by use of a SHARPLES centrifuge at a speed of about 40,000 R.P.M.; water flowed at rate of one litre per three minutes.

was found to be present in minute but measurable amounts, which were always greater near the bottom than at the surface.

Previous estimations of nitrate in sea water had been carried out by reducing it to ammonia and either treating with NESSLER solution as usual or estimating the nitrogen evolved (GAD-ANDRESEN 1923). HARVEY, however, made use of DENIGÈS (1910) qualitative test with reduced strychnine and proved that it was capable of giving quantitative results when the colour given by each batch of the reagent is compared with a standard. Numerous analyses were made by this means both of the water of the English Channel and from the depths of the Atlantic, mercuric chloride being used as a preservative. Table 9 has already shown how rich in nitrate are the dark depths of the ocean. The upper 50 metres are greatly depleted and average slightly under 10 mg. of nitrate nitrogen per m³, the phosphate, however, has been completely used up within this layer of water. Very similar results were obtained in the water off Norway and in the Faroe-Shetland Channel, save that in these northern waters neither nitrate nor phosphate had been entirely removed.

Thus, just as was found for phosphate, the deep ocean water acts as a reservoir of nitrate, which is regenerated at or near the bottom both in the ocean and in shallower seas. Table 12 records the condition at the three stations E 1, 22 miles from Plymouth Hoe on the course to Ushant, E 2 in mid-channel and E 3 off Ushant.

Table 12. Nitrate content as N_2 in mg. per m³, during 1925.

m.	May 13th			July 8th			Dec. 11th & 15th	
	E 1	E 2	E 3	E 1	E 2	E 3	E 1	E 2
0	2	—	67	4	3[1])	40[1])	38	—
10	3	55	85	—	—	—	40	106
20	5	—	85	4	24[2])	40[2])	43[2])	120[2])
30	2	53	67	—	—	—	—	—
50	9	15	67	—	29	40	48	120
70	8	—	—	4	—	—	43	—
90	*	29	—	*	29	—	*	132
105	*	*	67	*	*	40	*	*

The seasonal changes in nitrate at Station E 1 are shown in Fig. 19 and their similarity to those in which phosphate is concerned (Figs. 11

[1]) 5 metres value.   [2]) 25 metres value.

and 12) is at once evident. In the open sea then phosphate and nitrate are both completely used up by the phytoplankton, though a closer comparison of the results shows that sometimes one and sometimes the other appears to be the limiting factor. Too much stress cannot, however, be laid upon small differences in figures representing, very approximately, parts per thousand million. In Table 13 the data for both salts have
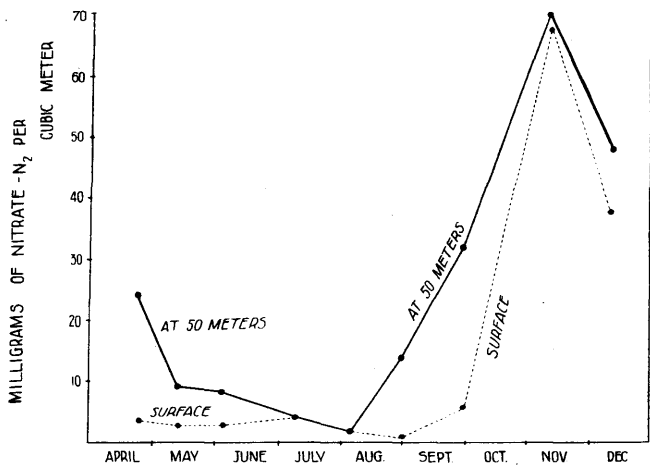

Fig. 19. Nitrate content of sea water at Station E 1, 22 miles S.W. of Plymouth, during 1925 in the surface 5 metres and at 50 metres.

been arranged so that a comparison may be made of the conditions prevailing at such periods. It should, however, be added that owing to the differences between successive batches of the reagent and the shorter column of liquid examined the accuracy obtainable in the nitrate estimations is possibly less than in those of the phosphate.

A study of Table 13 makes it clear that, though as regards the surface water in April, May and July phosphate is less plentiful than nitrate, if we consider the upper 20 metres there is but little difference, and in June the surface values were identical. Viewing the water column as a whole one important fact makes itself evident, namely that there is a continuous decrease in nitrate up to Aug. 5th, when the whole column is almost entirely depleted. The value, however, is but little less than that of July 8th. But the phosphate has, during the period from April to August, shown marked variations, a rise on June 3rd and a greater

Table 13. Phosphate as P_2O_5, shown under P, and nitrate as N_2 under N, both in mg. per m³, as found in sea water at Station E 1 during the spring and summer of 1925.

m.	Apl. 22nd		May 13th		June 3rd		July 8th		Aug. 5th		Aug. 31st		Oct. 1st	
	P	N	P	N	P	N	P	N	P	N	P	N	P	N
0	1	4	1	2	3	3	0	4	5	0	0	0	8	6
10	3	2	5	3	104	3	5	—	7	—	2	—	11	6
20	5	3	7	5	12	—	5	4	16	3	6	9¹)	17	21
30	—	—	11	2	17	—	7	—	24	—	6	—	19	32
50	13	24	—	9	21	8	9	4	—	3	—	14	20	32
70	16	24	12	8	21	9	10	4	24	0	5	14	21 (19²)	

one on Aug. 5th; it thus appears that phosphate is regenerated during this portion of the year more rapidly than is nitrate, accordingly the absence of nitrate early in August allowed of a considerable accumulation. Later on, however, nitrate is regenerated more rapidly than phosphate. The probable explanation is as follows; the regeneration of phosphate is for the most part simply the splitting up of organic compounds in which the phosphate radicle is present as such, though a portion is present in more complex compounds and in the insoluble and aggregated condition of calcium phosphate in exoskeletons and bones. Nitrate production is, however, the last of a series of changes, so there is of necessity a latent period while the protoplasm is being broken up to ammonium salts, which in turn are converted into nitrates. Thus it follows that during the latent period for nitrate an accumulation of phosphate takes place, which, however, is soon overtaken by nitrate formation. This appears to be the interpretation to be placed on the figures for Aug. 31st, in which much of the regenerated nitrate has been used up in producing phytoplankton sufficient to deplete the phosphate to the lowest value found during three years. At the surface the supply of both has been exhausted. By Oct. 1st the nitrate has established a definite lead in the deeper water; this had become far greater in November, in which the concentrations were: phosphate 25, nitrate 68 mg. per m³ in the surface water.

HARVEY has further shown that ammonium salts added to surface water from E 1 give rise to no nitrate, whereas when bottom water is used the production of nitrate can be demonstrated inside less than a

¹) 25 metres value.

²) Low owing to analytical error caused by reducing action of abundant organic matter.

week. If, however, *debris* from the bottom be added to E 1 surface water, it too produces nitrate — evidently because the requisite bacteria have been supplied.

Oxidation in natural waters.

The breaking down of living matter and of cellulose plant cell walls in the sea has not as yet received much attention. The total proteid may be deduced approximately from the figure for albumenoid ammonia. BRANDT's (1920) determinations and a certain number carried out in the Government Chemist's Laboratory, London, are available. The latter were in connection with questions concerning oyster mortality and are for inshore waters.

An idea of the amount of respirable organic matter in sea water was obtained (1922) by noting the change produced in the pH value on storing after addition of the indicator. In spring but little alteration occurs, whereas in autumn the reverse is true. At the levels between 15 and 25 metres and again near the bottom, marked differences are observed. In the production of these the organic matter of such of the plankton as die during storage is concerned.

The pH decrease observed corresponds to the production of 1.0 to 2.5 or 3.0 c.c. of $N/_{100}$ acid per 100 c.c. of sea-water. This is equivalent to the carbonic acid produced by the complete oxidation of from 3 to 8.0 or 9.6 mg. of a hexose sugar per litre. Determinations by RABEN on freshly drawn filtered sea water show an absorption of 8 mg. of oxygen per litre, as determined by the alkaline permanganate method. This corresponds to 7.5 mg. p. l. of hexose sugar. The writer (1923) has, however, found much lower values, about 1 mg. p. l. of oxygen absorbed, for water at E 1; this, however, was in March, when but little unoxidised matter is to be expected.

Oxidation in sea water has been studied by HARVEY (1925) and it was proved that certain easily oxidised substances were oxidised more readily by the deeper water at E 1 than by the surface water. There are indications of the presence of a catalyst, probably an iron compound. Its action is inhibited in surface and inshore waters by dissolved organic matter. Evidence afforded by a quantitative study of the rate of destruction of hydrogen peroxide by sea water confirms the foregoing.

Conclusion.

From the foregoing survey certain facts emerge. To begin with, there are as yet many lacunae in our knowledge of algal metabolism

and its seasonal changes. For one thing, further work is required upon the ammonium compounds in sea water. It has perhaps been tacitly assumed, though quite unintentionally, that ammonium salts are not directly utilized by algae — they have in fact been regarded as a stage in the production of nitrate. That even the higher plants can use them has been proved by OLSEN (1923), who showed that provided the production of excessive acidity is avoided, ammonium salts and nitrates can both be used.

Again, owing to the variations in the distribution of the plankton it is impossible to form a direct estimate of the seasonal crop. Calculations based upon the changes in the minor chemical constituents of the water afford, however, an estimate which is trustworthy provided the conditions which limit their applicability be borne in mind.

Furthermore a mass of evidence has been produced in support of NATHANSOHN'S (1906) views regarding the importance of the vertical circulation of the water in connection with the production of an abundant plankton.

Of the minor constituents studied so far it has been established that phosphate and under certain conditions nitrate may limit the production of the phytoplankton.

In conclusion the writer desires to express his thanks to Prof. H. H. DIXON and to Dr. H. H. POOLE for their help in revising this paper, also to Dr. E. J. ALLEN and Mr. H. W. HARVEY for assistance with the literature of the subject and to the Marine Biological Association of the United Kingdom and the Royal Dublin Society for the loan of the blocks for the figures.

Summary.

The action of the phytoplankton in removing carbon dioxide from sea water results in an increase in alkalinity in the surface 20 metres; this becomes extended to the bottom of the English Channel in October owing to vertical mixing. The amount of photosynthesis is equivalent to one kilogram of glucose in a water column 83.3 m. deep with surface area four square metres.

The phosphate content of sea water decreases in spring and increases in autumn. In the English Channel at Station E 1, depth 70 m., the annual consumption is close to 30 mg. P_2O_5 per m³, corresponding to the production of 1.4 kg. of phytoplankton, wet weight, for the one square metre water column, or 1,400 metric tons per sq. kilometre. Assuming a 15 per cent. carbohydrate content the alkalinity measure-

ments also indicate 1,400 tons; the exact agreement is fortuitous but the methods afford mutual support. In spring all the surface phosphate is used up and as the year advances but little is left at the bottom in the English Channel. Lack of phosphate apparently limits the production of phytoplankton in spring, and later in summer lack of nitrate may also set a limit. Both are regenerated ceaselessly and accumulate when light is insufficient for rapid growth. The dark ocean depths sewe as store-houses for these nutritive salts.

There is no proof that silica is ever a factor limiting diatom production in the ocean, though in spring the small amount present becomes much reduced.

In certain fresh waters the variations in the salts necessary for plant growth are much greater than in the sea.

The data presented furnish reasons for the proved richness of n orthern and coastal waters and support NATHANSON's views.

Bibliography.

Part II. Section on carbon dioxide assimilation.

ATKINS, W. R. G. 1922. The hydrogen ion concentration of sea water in its relation to photosynthetic changes. Part. 1, Journ. Marine Biol. Assoc., *12*, No. 4, 717—771.

— 1923. Part. 2, *Loc. cit.*, *13*, No. 1, 93—118.

— 1924. Part 3, *Loc. cit.*, *13*, No. 2, 437—446.

— and HARRIS, G. T. 1924. Seasonal changes in the water and heleoplankton of fresh-water ponds. Sci. Proc. Roy. Dublin Soc., *18*, 1—21.

— and HARVEY, H. W. 1925. The variation with depth of certain salts utilized in plant growth in the sea. Nature, *116*, 784.

BIRGE, E. A. and JUDAY, C. 1911. The inland lakes of Wisconsin. The dissolved gases of the water and their biological significance. Wisconsin Geol. and Nat. Hist. Survey. Madison, Wis.

CHAMBERS, C. O. 1912. The relation of algae to dissolved oxygen and carbon dioxide, with special reference to carbonates. 23rd Ann. Report, Missouri Bot. Gardens, 171—207.

GAARDER, T. 1917. Die Hydroxylzahl des Meerwassers. Bergens Mus. Aarb. Pp. 115.

HELLAND-HANSEN, B. 1914. Eine Untersuchungsfahrt im Atlantischen Ozean mit dem Motorschiff "Armauer Hansen" im Sommer 1913. Internat. Rev. d. ges. Hydrobiol. u. Hydrogr. *7*, 61.

KANITZ, A. 1915. Temperatur und Lebensvorgänge. Berlin.

KROGH, A. 1904. On the tension of carbonic acid in natural waters and especially in the sea. Meddelelser om Grönland, *26*, 333.

LEGENDRE, R. 1925. La concentration en ions hydrogène de l'eau de mer. Paris.

MATTHAEI, G. L. C. 1905. Experimental researches on vegetable assimilation and respiration. III. On the effect of temperature on carbon dioxide assimilation. Phil. Trans. Roy. Soc. B. *197*, 47—105.

McClendon, J. F. 1917. The standardisation of a new colorimetric method for the determination of the hydrogen ion concentration, CO_2 tension, and CO_2 and O_2 content of sea water etc. J. Biol. Chem., *30*, 265—88.

Moore, B., Prideaux, E. B. R., and Herdman, G. A. 1915. Studies of certain photosynthetic phenomena in sea water. Proc. & Trans. Liverpool Biol. Soc., *29*, 233—64.

Palitzsch, S. 1911. Sur le mesurage et la grandeur de la concentration en ions hydrogène de l'eau salée. C. R. Lab. Carlsberg, *10*, 54.

— 1912. Measurement of the hydrogen ion concentration in sea water. Rep. on Danish Oceanographical Exped. *1*, 237.

Section on phosphate.

Allen, E. J. 1919. A contribution to the quantitative study of plankton. Journ. Marine Biol. Assoc., *12*, 1—8.

Atkins, W. R. G. 1923. The phosphate content of sea-water in relation to the growth of the algal plankton. Part 1. Journ. Marine Biol. Assoc., *13*, No. 1, 119—150.

— 1925. Part 2. *Loc. cit.*, *13*, No. 3, 700—720.

— 1926. Part 3. *Loc. cit.*, *14*, No. 2, forthcoming.

Brandt, K. 1920. Ueber den Stoffwechsel im Meere. 3 Abhandlung. Wiss. Meeresuntersuch., Abt. Kiel, *18*, 185—430.

Denigès, G. 1921. Détermination quantitative des plus faibles quantités de phosphates dans les produits biologiques par la méthode céruléo-molybdique. C. R. Soc. Biol. Paris, *84*, No. 17, 875—877. Also C. R. Acad. des Sc., Paris, 1920, *171*, 802.

Jespersen, P. 1924. On the quantity of macroplankton in the Mediterranean and the Atlantic. Internat. Rev. d. ges. Hydrobiol. u. Hydrogr., *12*, Hft. 1—2, 102—115.

Lohmann, H. 1920. Die Bevölkerung des Ozeans mit Plankton u. s. w. Archiv f. Biontologie, *4*, Heft 3, S. 1—617.

Matthews, D. J. 1916. On the amount of phosphoric acid in the sea water off Plymouth Sound. Journ. Mar. Biol. Assoc., *11*, 122—130.

— 1917. Pt. 2. *Loc. cit.*, *11*, 251—257.

Pouget, L., and Chouchak, D. 1909, 1911. Dosage colorimétrique de l'acide phosphorique. Bull. Soc. Chim. France, Series 4, *5*, 104 and *9*, 649.

Raben, E. 1920. Quantitative Bestimmung der im meerwassergelösten Phosphorsäure. Wiss. Meeresuntersuch., Abt. Kiel, *18*, 1—24.

Section on silicate.

Atkins, W. R. G. 1923. The silica content of some natural waters and of culture media. Journ. Mar. Biol. Assoc., *13*, No. 1, 151—159.

Atkins, W. R. G. 1926. Seasonal changes in the silica content of natural waters in relation to the phytoplankton. *Loc. cit.*, *14*, No. 1.

Brandt, K. 1920. As in phosphate section.

Wells, R. C 1922. Determination of silica in filtered sea-water. J. Amer. Chem. Soc., *44*, 2187—93.

Section on nitrogen compounds, oxidisable matter and conclusion.

Alsterberg, G. 1924. Die Bestimmung von Nitrit und Nitrat mit besonderer Berücksichtigung hydrobiologischer Verhältnisse. Bot. Notiser, Lund, 279—283.

ATKINS, W. R. G. 1922. The respirable organic matter of sea water. Journ. Mar. Biol. Assoc., *12*, No. 4, 772—780.

— 1923. Note on the oxidisable organic matter of sea water. *Loc. cit.*, *13*, No. 1, 160—163.

— and HARVEY, H. W. 1925. As in assimilation section.

BRISTOL, B. M., and PAGE, H. J. 1923. A critical enquiry into the alleged fixation of nitrogen by green algae. Ann. Applied Biol., *10*, 378—408.

BRANDT, K. 1920. As in phosphate section.

BUCH, K. 1915. Bestimmungen des Ammoniak-Gehaltes im Wasser der Finnland umgebenden Meere. Finska Vetenskaps-Soc. Förhandl., *57*, Afd. A., No. 21, pp. 15.

— 1923. Methodisches über die Bestimmung von Stickstoff-Verbindungen im Wasser. Havsforskningsinst. Skrift No. 18, pp. 22.

DENIGÈS, G. 1911. Determination of nitrates and nitrites in water by means of reduced strychnine. Bull. Soc. chim., (IV), 544—546, from J. Chem. Soc., 1911, A 11, 655.

DOMOGALLA, B. P., JUDAY, C. and PETERSON, W. H. 1925. The forms of nitrogen found in certain lake waters. J. Biol. Chem., *63*, 269—285.

DREW, A. H. 1914. The awakening of pond life in the spring. Science Progress, *9*, No. 33, 96—104.

GAD-ANDRESEN, K. L. 1923. A method for quantitative determination of ammonia, nitrate and nitrite, together with other nitrogenous compounds, in sea water. Publ. de Circonstance No. 82, Conseil perm. internat. pour l'exploration de la mer.

HARVEY, H. W. 1925. Oxidation in sea water. Journ. Mar. Biol. Assoc., *13*, No. 4, 953—969.

— 1926. Nitrate in the sea. *Loc. cit.*, *14*, No. 1.

JUDAY, C. 1925. See Domogalla, also PETERSON.

NATHANSOHN, A. 1906. Ueber die Bedeutung vertikaler Wasserbewegungen für die Produktion des Planktons im Meere. Abhand. Kgl. Sächs. Ges. d. Wiss. math.-phys. Kl., *29*, No. 5, 355—441.

OLSEN, C. 1923. Studies on the hydrogen ion concentration of the soil and its significance to the vegetation, especially to the natural distribution of plants. C. R. Lab. Carlsberg, *15*, No. 1, 1—166.

ORR, A. P. 1926. The nitrite content of sea-water. Journ. Mar. Biol. Assoc., *14*, No. 1.

PETERSON, W. H., FRED, E. B., and DOMOGALLA, B. P. 1925. The occurence of amino acids and other organic nitrogen compounds in lake water. J. Biol. Chem., *63*, 287—295.

SCHRAMM, J. R. 1914. The relation of certain grass green algae to elementary nitrogen. Ann. Missouri Bot. Gardens, *1*, 2.

WANN, F. B. 1921. The fixation of nitrogen by green plants. Amer. Journ. of Bot., *8*, 1—29.

WITTING, R. 1914. Zur Methodik der Bestimmung von geringen Ammoniakmengen mit besonderer Berücksichtigung der Meerwasseranalysen. Finska Vetenskaps-Soc. Förhandl., *56*, Afd. A, No. 15.