

# The Phosphate Content of Fresh and Salt Waters in its Relationship to the growth of the Algal Plankton.

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With Figures 1-8 in the Text.

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## INTRODUCTION AND PHOSPHATE CONTENT OF FRESH WATER SUPPLIES.

ON account of the minute quantities in which they are present and of the fact that they are considered of secondary importance as indicating sewage contamination, phosphates are not usually estimated in analyses of natural waters. The tediousness of the determination also militated against it in the past. As a result, of the numerous analyses recorded by Clarke (1920), but few mention phosphates. C. H. Stone's analysis of the Mississippi in 1905, carried out upon a sample above Carrolton, Louisiana, shows 0.27 per cent of phosphate ( $\text{PO}_4$ ) with a total salinity of 146 parts per million, or 0.39 mgrm.  $\text{PO}_4$  per litre, corresponding to 0.29 mgrm.  $\text{P}_2\text{O}_5$ .

The presence of as little as 0.5 part  $P_2O_5$  per million, viz. 0.5 mgrm. per litre, is considered as indicative of sewage contamination (Kenwood, 1911, quoting Hehner), though owing to the rapid removal of phosphates by plants a smaller amount need not necessarily prove the purity of the water. The American Public Health Association's Standard Methods for water analysis do not include one for phosphate (1920).

Recently McHargue and Peter (1921) have carried out a large number of phosphate determinations in small and large streams and some of the great rivers of the United States. Spring water in an Ordovician area was found to contain 0.5–0.8 parts per million of phosphate as pentoxide; springs in other areas were considerably poorer, containing only 0.1–0.2 p.p.m. Figures for the rivers Ohio, Tennessee, Green River, Cumberland, Missouri, and Mississippi averaged 0.2 p.p.m. Calculating from the mean annual volume of the Mississippi near its mouth these authors conclude that the amount of the element phosphorus carried to the sea in solution amounts each year to 62,188 tons; to this must be added the phosphorus (0.15 per cent) in 7469 million cubic feet of suspended matter. The concentration of phosphate in the sea is, as will be shown later, far less than 0.2 p.p.m., so, while diluting the general salinity of the ocean, the river raises its concentration as regards phosphates.

In view of the scanty data available as to the quantity of phosphate in natural waters and reservoirs in this country, the following miscellaneous determinations carried out by the writer may be placed on record. The analyses were made by the colorimetric method of Denigès, as described later.

In order the better to characterize the water the pH value and electrical conductivity, which gives an idea of the proportion of total solids, are also tabulated (see Table I).

It may be seen that the phosphate content of uncontaminated streams and fresh water supplies is extremely small in the districts examined, being under 0.05 parts of  $P_2O_5$  per million. These values are considerably below those of McHargue and Peter, obtained in the U.S.A. How small these quantities are may be appreciated from the fact that Matthews (1916–18), when making up artificial sea water from the purest chemicals of Merck and Kahlbaum, found that the mixture contained 0.0286 mgrm. of  $P_2O_5$  per litre, and the writer has found hydrogen peroxide sold as free from phosphoric acid to contain the equivalent of 0.20 mgrm. of  $P_2O_5$  per litre.

The earlier analyses of the phosphate content of sea water are reviewed by Matthews (1916), Raben (1920), and Brandt (1920).

With samples taken just outside Plymouth Breakwater Matthews found a maximum of 0.06 mgrm. per litre at the end of December, 1915, with an irregular fall to a minimum of less than 0.01 in April and May.

He attributes the seasonal variation to the removal of phosphates from solution by the fixed algæ, the diatoms, and Phæocystis.

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 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 from various depths down to 800

TABLE I.

Source of water.	Phosphate as mgrms. $P_2O_5$ per litre.	Electrical conductivity at $0^\circ C. \times 10^6$ .	pH.
Plymouth tap, May . . . . .	0.003	26	6.6
Maryfield (Cornwall) tap, June . . . . .	0.023	270	7.2
Basingstoke tap, June . . . . .	0.032	270	7.2
Peverell (Plymouth) old reservoir, June . . . . .	0.278	222	—
Pool in waterlogged pasture, Anglesey . . . . .	0.167	290	6.9
Stream, Bodorgan, Anglesey, February . . . . .	0.019	192	6.8
Stream, basalt district, S. Scotland, March . . . . .	0.007	59	6.4
Ditch, calcareous sandstone district, S. Scotland, March . . . . .	0.016	186	6.9
Stream, S. Scotland, March . . . . .	0.021	72	6.8
Stream, Yorkshire, March . . . . .	0.036	227	7.1
Stagnant ditch, meadow, near Plymouth . . . . .	0.019	213	7.7
Ditch in lane, near Plymouth . . . . .	0.047	294	7.6
Yard well, Antony, Cornwall . . . . .	1.25	227	6.4
Sea water, winter . . . . .	0.049	28,200	8.1
Aquarium tanks, Plymouth . . . . .	4.81	30,300	7.6

metres in the North Atlantic. There is usually a considerable increase from the surface downwards. None of the values, however, indicate exhaustion of the water as regards phosphate, the minimum recorded figure being 51 mgrm. of  $P_2O_5$  per cubic metre (viz. 0.051 mgrm. per litre) and the maximum 221 mgrm., both values being from North Sea Station N7. These figures are about four times as great as those given by Matthews, whose results it may be added agree well with those obtained by the Government chemist, London, using the same method as Matthews upon samples sent from Plymouth in 1922, and with analyses carried out by the writer, according to an entirely different method.

In view of the importance of phosphates for plant growth it seemed of interest to make a further study of these seasonal changes, both in the sea and in fresh water, and to study the diminution of phosphate in laboratory cultures.

#### THE UPTAKE OF PHOSPHATE IN A DIATOM CULTURE.

A culture of *Nitzschia closterium* W. Sm., pure save as regards the presence of bacteria, was kindly supplied by Dr. E. J. Allen. This was growing in sea water enriched with Miquel's solution, as described by Allen and Nelson (1910). It was exposed in a north window for periods as given in Table 2, the temperature being about 12°–15° C. The results are shown in Fig. I, and it may be seen that a great increase in diatoms results in the almost complete utilization of the phosphate, which appears to be the factor limiting further multiplication.

TABLE II.

Changes in phosphate in culture flask of *Nitzschia closterium*.

Date.	Days.	Nitzschia, thousands per c.c.	P <sub>2</sub> O <sub>5</sub> as milligrams per litre.	—	—
17/3	0	0	—	—	—
27/3	10	510	2.38	—	—
13/4	26	2140	0.55	—	—
26/4	40	3065	0.006	—	—

From the count of 13/4 and the previous one  $1630 \times 10^6$  diatoms use up 1.83 mgrm. P<sub>2</sub>O<sub>5</sub>, namely,  $1 \times 10^9$  require 1.12 mgrm. From the final count  $925 \times 10^6$  diatoms have appeared at the expense of 0.544 mgrm., which is equivalent to 0.59 mgrm. per  $1 \times 10^9$  diatoms. This being considerably less, about half, the former value indicates either a reduction in size of the diatoms, which may result from their mode of division, or else a regeneration of phosphate from the protoplasm of dead diatoms; the hæmacytometer count includes all diatoms, but the number given may not all be alive.

An attempt was made to settle this point by estimating the phosphate content of a known number of diatoms. Accordingly 105 c.c. of *Nitzschia* culture was filtered through close-grained paper, and evaporated to dryness with hydrochloric acid, in order to decompose organic compounds containing phosphoric acid. The residue was then taken up with water, and since the culture contained  $2.9 \times 10^6$  diatoms per c.c., as read from the graph for the date of the analysis, it was ascertained that 0.307 mgrm. of P<sub>2</sub>O<sub>5</sub> was yielded by  $1 \times 10^9$  diatoms. Another portion

of the culture was taken later on, and submitted to the more drastic treatment of evaporation to dryness with nitric acid. The residue was then evaporated to dryness after having been taken up with water, and, finally, after the addition of sulphuric acid. The culture at this stage contained  $3.06 \times 10^6$  diatoms per c.c. and 0.303 mgrm.  $P_2O_5$  per  $1 \times 10^9$  diatoms was obtained, which agrees closely with the first analysis. Since the amount is, however, only about one-fourth of that taken up by the production of this number of diatoms it appears that the treatment

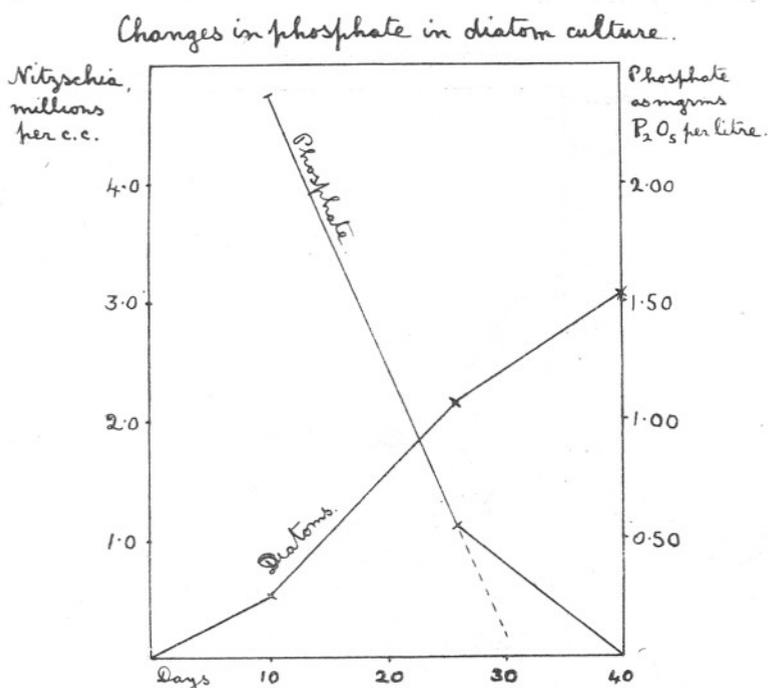


FIG. 1.

has not sufficed to convert all the organically combined phosphorus into the acid, but has split off a more easily hydrolysed fraction of it. The estimation of the total phosphorus has been deferred till a later date.

As  $1 \times 10^9$  of the diatom require 1.12 mgrm.  $P_2O_5$ , one gram of this should suffice for  $9 \times 10^{11}$  Nitzschias. It now becomes of interest to study the seasonal change in phosphate which occurs in sea water, and to estimate the Nitzschia crop that could be produced were the whole amount available for this organism, neglecting any processes that may enrich the sea with phosphate during its period of diminution.

THE DECREASE IN PHOSPHATE OCCURRING IN STORED SEA WATER WHEN  
INSOLATED.

Open sea water stored in the dark in bottles used for chloride samples, or in Winchester quart bottles, appears to undergo but little change for a couple of weeks in spring. There is, however, always the possibility that owing to the growth of moulds water kept for considerable periods may give low results, or even possibly high results, if bacterial decomposition has been active, though on the latter point there is as yet no direct evidence.

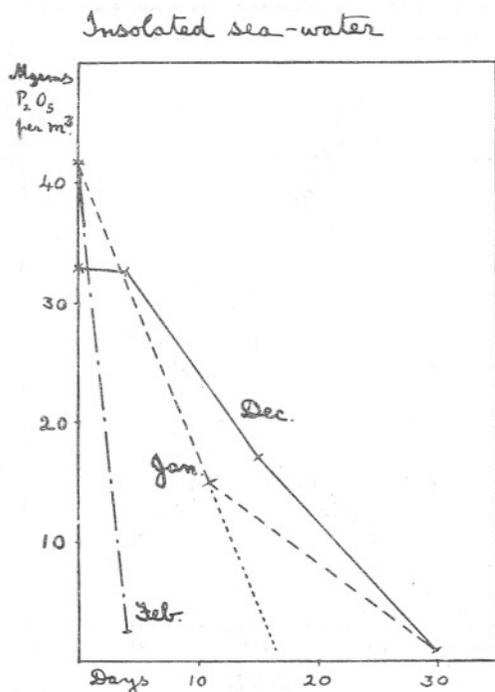


FIG. 2.

In order to test the hypothesis that the vernal decrease observed to occur in the sea was due to the uptake of phosphate by algæ, five Winchester quart bottles of water were exposed in a south window. Of these A and B were taken from Station E1 on December 18th and January 16th respectively, C and D at Station L5, the Eddystone, in quite similar sea water on February 12th and March 8th, whilst E was from L1 in Plymouth Sound. On insolation A and B decreased slowly at first, but only 0.001 mgrm. P<sub>2</sub>O<sub>5</sub> per litre was left after thirty days. The others, however, contained only 0.003 mgrm. per litre after four days. The results are shown in Table III and Fig. 2.

TABLE III.

Changes in phosphate content of stored sea water when insolated. Phosphate in milligrams of  $P_2O_5$  per cubic metre. Insolated from 24/3/23.

Sample	A	B	C	D	E
Source of water :	E1, 18/12/22.	E1, 16/1/23.	L5, 12/2/23.	L5, 8/3/23.	L1, 7/3/23.
Days.					
0	33	42	42	37	43
4	32.5	—	2.5	3	3.5
11	—	15	—	—	—
15	17	—	—	5	—
30	1	1	—	—	—

On account of their similarity to the line given by C, those for D and E have been omitted from the figure. It is evident that the diminution in phosphate becomes increasingly rapid as the spring samples are approached; the explanation appears to be that these contain a larger number of plankton algæ per unit volume, and their multiplication under the favourable light conditions speedily results in the consumption of the small amount of phosphate occurring in sea water. As many as 464 plankton organisms per cubic centimetre have been found by Allen (1919) in sea water in summer. In winter, however, the much smaller numbers present can apparently be doubled or quadrupled with but little effect upon the amount of phosphate as ascertained by analysis.

The figures obtained make it clear that, just as in the *Nitzschia* culture, which was artificially enriched with nitrates, in sea water also algal growth results in the uptake of phosphate till none remains, for a quantity such as 0.001 mgrm. per litre (viz. 1 in  $10^9$ ) is about the limit which can be detected by the extremely delicate method used. Recent work by Pentanelli (1923), of which an abstract only has been seen, claims to show that the development of marine algæ in unchanged sea water is stopped by deficiency in carbon dioxide, nitrogen, and phosphorus, and by an alteration of the water which is independent of the consumption of food.

In this connection it may be remarked that Allen and Nelson (1910) found that the tank water was more favourable, when sterilized, for the cultivation of diatoms than was open sea water. This is, no doubt, due in part at least to its higher phosphate content. It may also be added that the Laboratory supply of open sea water filtered through a Berkefeld candle, as explained by Allen and Nelson, was found, after standing in a covered beaker for a fortnight, to contain less than 0.01 mgrm.  $P_2O_5$  per litre, whereas water freshly drawn contained 0.12 mgrm. Sea water at the time had about 0.049 mgrm. When filtered through a Doulton filter candle, which had been well washed with tap water containing

under 0.02 mgrm.  $P_2O_5$  per litre, sea water was deprived of phosphate. After rejecting the first portion likely to be diluted by fresh water, the next 80 c.c. was found to have 0.020 mgrm. per litre. A further 300 c.c. gave 0.026 mgrm. None of the sea water analyses recorded by the writer were made upon filtered water unless expressly stated to the contrary.

As mentioned in the analytical section of this paper, Matthews used ferric chloride solution to precipitate the phosphate of sea water for estimation. It was found by the writer that on adding a few drops of Laboratory reagent ferric chloride all phosphate was removed with the ferric hydroxide precipitate and the filtered solution contained not more than 0.001 mgrm.  $P_2O_5$  per litre.

With water from the Aquarium tanks containing 4.75 mgrm.  $P_2O_5$  per litre the addition of ten drops of ferric chloride to a beaker containing about a litre reduced the phosphate to 0.62 mgrm. and the pH value from 7.6 to 6.7. A further ten drops brought the reaction to pH6.6 and the phosphate down to 0.01. On bringing the total number of drops up to thirty, a great increase in acidity, pH3.4, was found, together with an increase in the phosphate in solution. One drop of 0.880 ammonia, however, made the solution alkaline, about pH10, and reduced the phosphate to 0.005 mgrm. per litre. This action of iron in precipitating phosphate is of much biological importance, and should be considered when culture media are being prepared.

#### SEASONAL CHANGES IN PHOSPHATE IN SEA WATER, 1922 RESULTS.

Table IV shows seasonal variations of phosphate, expressed in milligrams of  $P_2O_5$  per litre; the analyses were carried out on surface samples stored for some weeks at the Government Chemists' Laboratory, London, by Pouget and Chouchak's colorimetric method, as used by Matthews.

TABLE IV.

Date.	L2 and L3.	E1.	E2.	E3.	N2.
12/2/22	0.051	—	0.070	—	0.016
15/3	—	0.046	—	—	—
30/3	0.034	0.039	—	—	0.041
25/5	—	—	—	0.022	0.031
6/6	0.012	0.015	—	—	—
12/7	—	0.019	—	0.020	0.019

Aquarium of the Marine Biological Association, east reservoir, about 5.0 mgrm. per litre.

As already mentioned, these results agree well with those obtained by Matthews in 1916, his site, the Knap Buoy, being in between stations L2 and L3. They further show that these changes occur simultaneously in the sea water over a wide area. It should be explained that the L series of stations extend from below the Laboratory, in Plymouth Sound to L6, which is half-way between the Eddystone (L5) and E1. The remainder are the International Hydrographic Stations, E1, E2, and E3, lying on the course from the Eddystone to Ushant, N1, N2, N4, and N5, on the course from Ushant to Cork Harbour. N3 is between the Scilly Islands and Cornwall, E6 being 20 miles to the north in the Bristol Channel. Their positions are shown in the map given by the writer (1922).

The relatively high value 5.0 mgrm. per litre given by the water of the Aquarium is noteworthy, as it indicates the mode, or one mode, whereby the phosphate taken up by the algal plankton is returned again to the sea—namely, through the excretion of phosphate by fish and marine invertebrates. The tanks are well stocked with both, but there is little algal life, so the normal balance of the sea is disturbed.

It may be added that similar values for the tank water have been obtained by the writer, viz. 4.75 mgrm. per litre for both east and west reservoirs on April 10th, 1923, and 4.81 mgrm. on June 29th. The reservoirs had been drained and refilled between these analyses and that of the Government chemist.

#### SEASONAL CHANGES IN PHOSPHATE AT L STATIONS, 1923.

The work was continued in 1923, all the determinations being made by the writer according to the method of Denigès, upon samples taken the same or the preceding day. The samples were kept in the dark during the interval.

Table V gives the results for the L series from March to August. Certain values for water taken at the east slip, directly below the Laboratory, are also included. Owing to sewage contamination these do not exhibit regular seasonal changes. The effect of sewage upon the L1 values is surprisingly small, judging by the uniformity of the figures with those of other stations. Low values were obtained from the end of April onwards, and Fig. 3 represents the seasonal change at L4, half-way between Rame Head and the Eddystone, about five miles outside the Breakwater.

Within the limits of experimental error the surface values are equal to or less than the bottom, due to the fact that photosynthesis and consequently algal growth and reproduction is more active near the surface. Occasionally, however, one meets with an abnormal surface

value, such as that for L4 on May 31st and L6 on August 15th. One can only attribute these results to a local contamination of the water from a ship, as the bucket had been rinsed repeatedly, as were also the bottles.

TABLE V.

Seasonal variations of phosphate, expressed in milligrams of  $P_2O_5$  per litre, surface samples mainly.

Date.	East slip.	L1.	L2.	L3.	L4.	L5.	L6.
7/3/23	0.0485	0.0485	0.049	0.049	—	—	—
12/3/23	—	0.049	0.045	—	0.041	0.033	—
21/3/23	—	0.042	0.040	0.041	—	0.038	—
22/3/23	0.0395	—	—	—	—	—	—
27/3/23	—	—	—	0.020*	—	—	—
28/3/23	—	0.032	0.033	0.039	0.037	—	—
9/4/23	—	0.033	0.033	—	—	—	—
9/4/23	—	0.036B	0.033B	—	—	—	—
11/4/23	—	—	—	—	—	0.031	—
11/4/23	—	—	—	—	—	0.041B	—
16/4/23	—	0.021	0.020	0.013	0.016	0.023	—
16/4/23	—	0.024B	0.024B	0.018B	0.028B	0.021B	—
18/4/23	—	—	—	0.024	—	—	—
18/4/23	—	—	—	0.024B	—	—	—
20/4/23	—	0.024	—	—	0.014	0.024	—
20/4/23	—	0.028B	—	—	0.027B	0.023B	—
24/4/23	—	0.016	0.010	0.015	0.021	0.023	—
3/5/23	0.042	—	—	—	—	—	—
7/5/23	—	0.027	0.025	—	0.023	—	—
7/5/23	—	—	—	—	0.023B	—	—
22/5/23	—	0.0235	0.0155	0.023	0.0105	0.012	0.004
31/5/23	—	—	—	0.0065	0.050†	—	—
31/5/23	—	—	—	0.0065B	0.046†	—	—
31/5/23	—	—	—	—	0.008B	—	—
19/6/23	—	0.0045	—	0.0055	—	0.004	—
19/6/23	—	0.009B	—	0.0115B	—	0.012B	—
23/6/23	0.008	—	—	—	0.009	—	—
2/7/23	—	—	—	—	0.013	—	—
2/7/23	—	—	—	—	0.013B	—	—
10 & 12/7/23	—	—	—	0.013	0.014	0.0135	0.007
10 & 12/7/23	—	0.017B	0.019B	0.017B	0.016B	0.014B	—
15/8/23	—	0.017	0.019	0.017	0.010	0.020	0.032
15/8/23	—	—	—	0.017B	0.021B	0.019B	0.020B

The general trend of the seasonal changes in the L series is illustrated in Fig. 3, in which are plotted the results for L4. The abnormal result for May 30th has been omitted, and the bottom value taken for surface also since L3 had identical values for both on that date; these differed

\* Mean of two samples. B indicates bottom sample.

† Abnormal result verified by analysis on two bottles.

only by 0.0015 from the L4 bottom value. The curve is similar to that obtained by Matthews, save that the seasonal changes are about a month later all through. Comparison with the bottom values shows how a low surface value in April may so quickly be followed by one over twice as great; clearly the deeper water acts as a reservoir of phosphate, as is more fully shown in subsequent figures. The higher bottom value found in August indicates that the regeneration of phosphate takes place in the deeper water, or rather that its effect is more evident there since it is being rapidly removed at the surface in summer.

It seemed possible that these changes could be detected in rock pools, exposed for several hours each tide.

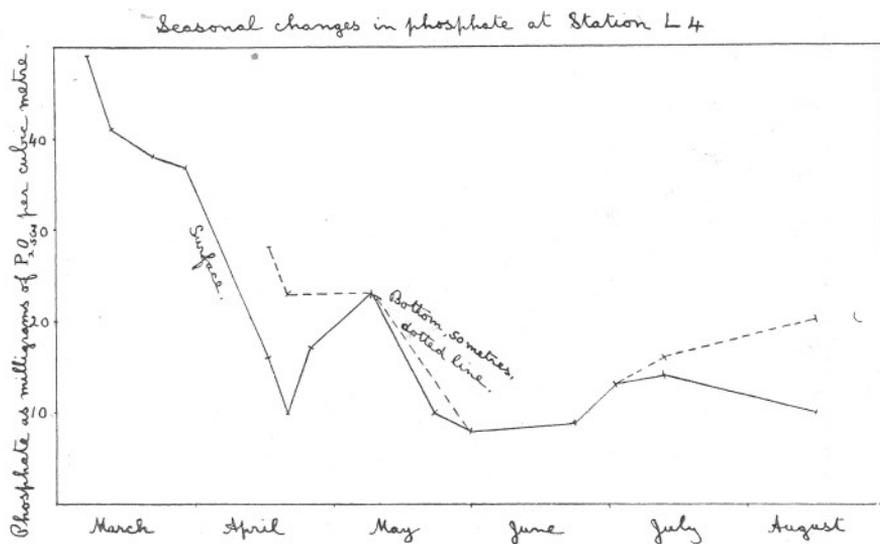


FIG. 3.

It was at first thought permissible to compare the pool water with that taken about a hundred yards to the east at the slip, but results given in Table VI (page 133) show that sewage contamination renders this unreliable. As far as the analyses go they indicate an increase in phosphate in the pools during their separation from the sea on two days, but an appreciable decrease one very sunny day. The pools have an abundance of animal life as well as algæ, so excretion may account for the small increases noted.

#### SEASONAL CHANGES IN PHOSPHATE AT THE INTERNATIONAL HYDROGRAPHIC STATIONS E1-E3 AND N1-N3.

Table VII (page 133) contains the results of the analyses of sea water taken at E1 from March to August at various depths. From the end of

May onwards the surface water may be seen to be almost totally devoid of phosphates. Fig. 4 makes this clear, and an increase in the phosphate content of the bottom water in August is also noticeable. Fig. 5 illustrates the variations in phosphate with depth; the seasonal change is here shown by the shifting of the curve to left for diminution or to right or increase. Bad weather precluded the taking of a February series, but the sea water was apparently richer in phosphate then than in March, judging from Matthews' results.

The differences which exist, in the calmer summer weather, between

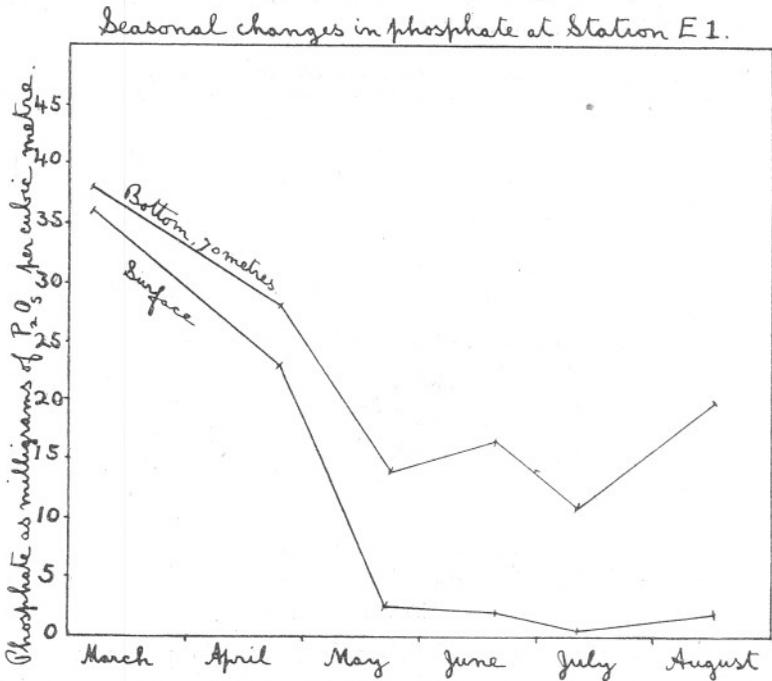


FIG. 4.

surface and deeper water samples show that mixing of the water is not rapid at E1 at this season. On account of the diminution in the intensity of the light the phosphate in the deeper water is not used up till it is brought nearer the surface, or at least it is used up at a greatly reduced rate.

In Table VIII (page 134) the corresponding data are given for Stations E2 and E3. The depth series results are plotted in Fig. 6 (page 132). Samples taken on the cruises to Ushant, etc., have perforce to await analysis for two to three days, but no appreciable error appears to be introduced by this as the samples are stored in the dark.

The almost total depletion of the phosphate down to 10 metres is noticeable at E2, and here, as at E1, the minimum value is found in July. At E3, however, the May value is the lowest for the bottom, and the mixing of the water diminishes the surface to bottom gradient.

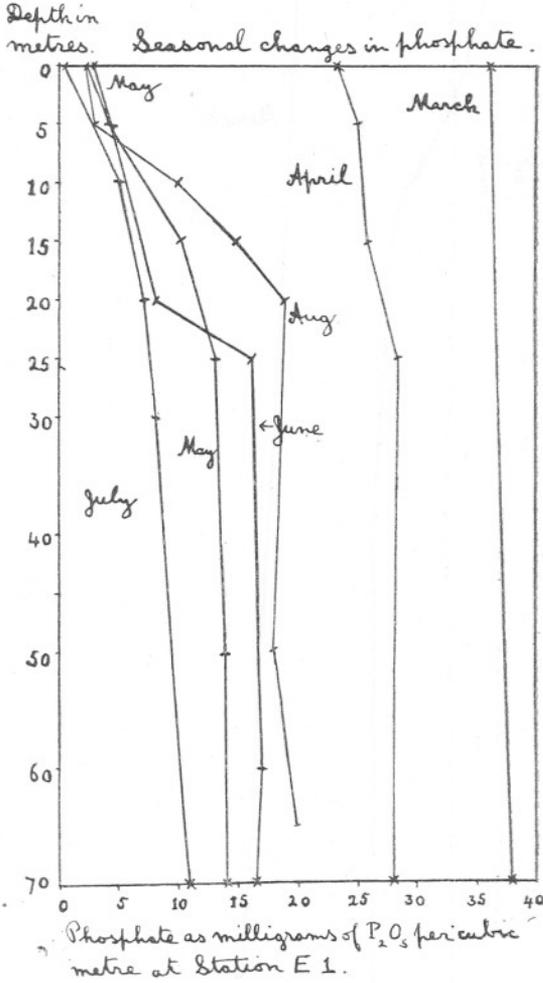


FIG. 5.

This has also been observed as regards temperature and pH gradients at this station, as pointed out by the writer in an accompanying paper in this Journal.

For Stations N1, N2, and N3 no April records are available owing to the renewal of stormy weather during the cruise, and a thick fog

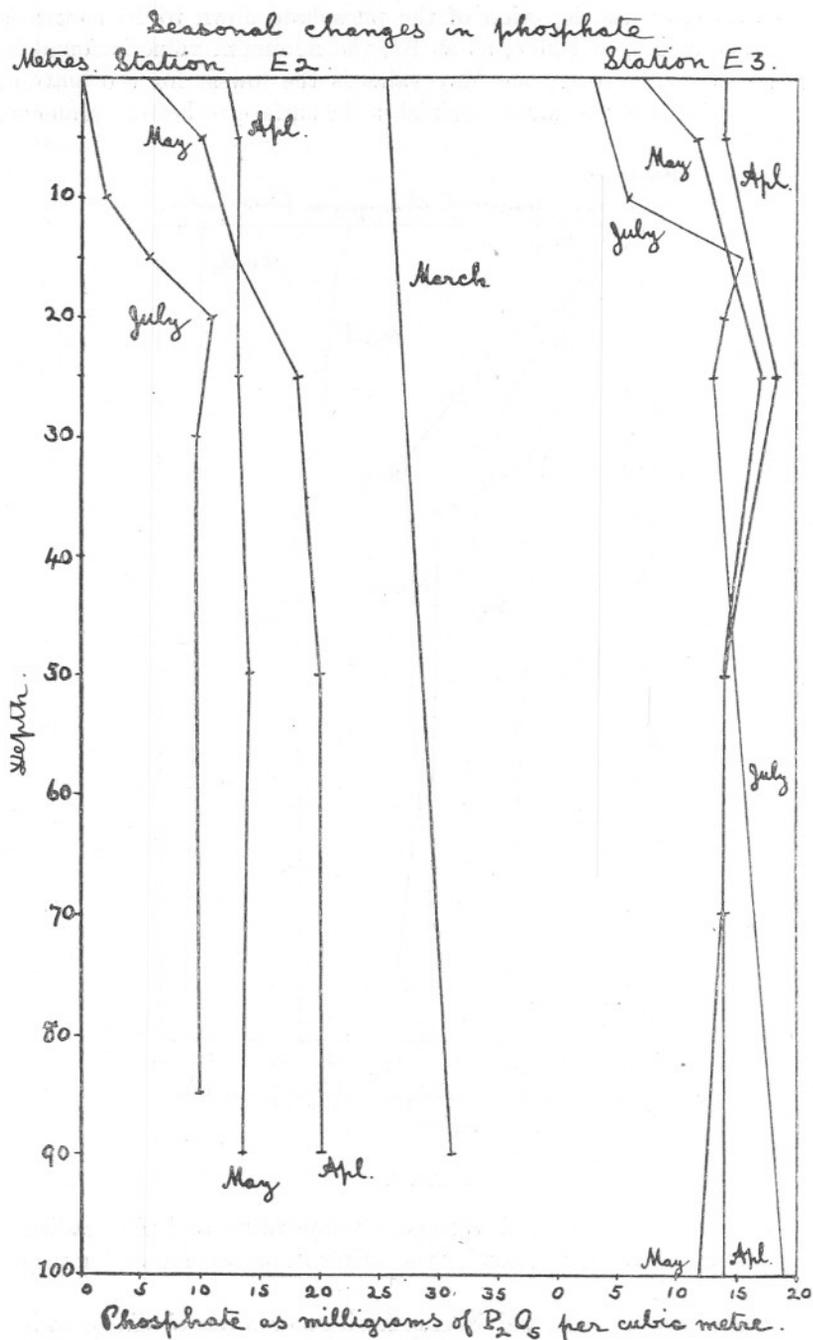


FIG. 6.

prevented the obtaining of samples at N3 in July. The analyses for May and July are given in Table IX (page 134) and plotted in Fig. 7 (page 135). At both N1 and N2 the July values are greater than those for May in the samples from the deeper water. The gradient is also very remarkable, especially the difference between the 15- and 20-metre samples at N1. The settled calm weather appears to account for this. There is a suggestion of regeneration of phosphate at both N1 and N2, or the higher values may be due to a transgression of deeper water moving eastwards over the edge of the west European submarine shelf.

TABLE VI.

Source of water.	Date.	Hour.	Phosphate as mgrms. $P_2O_5$ per litre.	Notes
East slip . . .	3.4.23	11.40 a.m.	0.031	
Rock pool west of slip . . . „		11.50 a.m.	0.037	
Do. . . „		2.50 p.m.	0.040	
East slip . . .	4.4	11 a.m.	0.033	
Do. . . „		5 p.m.	0.0325	
Rock pool . . . „		12.50 p.m.	0.036	
Sound, by pool . . . „		„	0.036	
Another pool, close to first one . . . „		4 p.m.	0.040	First pool submerged.
East slip . . .	5.4	10.15 a.m.	0.039	
Rock pool . . . „		12.30 p.m.	0.039	Pool covered at
Sea by pool . . . „		„	0.039	10.30 a.m.
Pool . . . „		4.15 p.m.	0.030	Very sunny day.
East slip . . . „		4.15 p.m.	0.055	Sewage effect.

TABLE VII.

Seasonal variations of phosphate, expressed in milligrams of  $P_2O_5$  per litre, Station E1.

Depth in metres.	March 7th	April 24th.	May 22nd.	June 19th.	July 10th.	August 15th.
0	0.036	0.023	0.0025	0.002	0.0005	0.002
5	—	0.025	0.004	0.004	—	0.003
10	—	—	—	—	0.005	0.010
15	—	0.026	0.010	—	—	0.015
20	—	—	—	0.008	0.007	0.019
25	—	0.0285	0.013	0.016	—	—
30	—	—	—	—	0.008	—
50	—	—	0.014	—	—	0.018
60	—	—	—	0.017	—	—
70	0.038	0.028	0.014	0.0165	0.011	0.020

TABLE VIII.

Seasonal variations of phosphate, expressed in milligrams of  $P_2O_5$  per litre, Stations E2 and E3.

Depth in metres.	March 14th.	April 24th.	May 22nd.	July 10th.	April 25th.	May 22nd.	July 10th.
0	—	0.012	0.0055	0.000	0.014	0.007	0.003
5	0.0255	0.013	0.010	—	0.014	0.0115	—
10	—	—	—	0.002	—	—	0.006
15	—	0.013	0.013	0.0055	—	—	0.0155
20	—	—	—	0.011	—	—	0.014
25	—	0.018	0.013	—	0.0185	0.017	0.013
30	—	—	—	0.0095	—	—	—
40	—	—	—	—	—	—	—
50	—	0.020	0.014	—	0.014	0.014	—
60	—	—	—	—	—	—	—
70	—	—	—	—	—	0.014	—
80	—	—	—	0.010	—	—	—
90	0.031	0.020	0.0135	—	—	—	—
100	—	—	—	—	0.014	0.012	0.019

TABLE IX.

Seasonal variations of phosphate, expressed in milligrams of  $P_2O_5$  per litre, Stations

Depth in metres.	N1		N2.		N3.
	May 22nd.	July 11th.	May 22nd.	July 11th.	May 22nd.
0	0.015	0.0045	0.017	0.014	0.016
5	0.016	—	0.015	—	—
10	—	—	—	—	—
15	0.013	0.005	—	0.014	—
20	—	0.022	—	0.015	—
25	0.016	0.021	0.015	0.0235	0.016
30	—	—	—	—	—
40	—	—	—	—	—
50	0.017	—	0.016	—	—
60	—	—	—	0.0235	0.0205
70	0.017	—	—	—	—
80	—	—	—	—	—
90	—	0.023	0.022	—	—
105	0.019	—	—	—	—

Seasonal changes in phosphate.

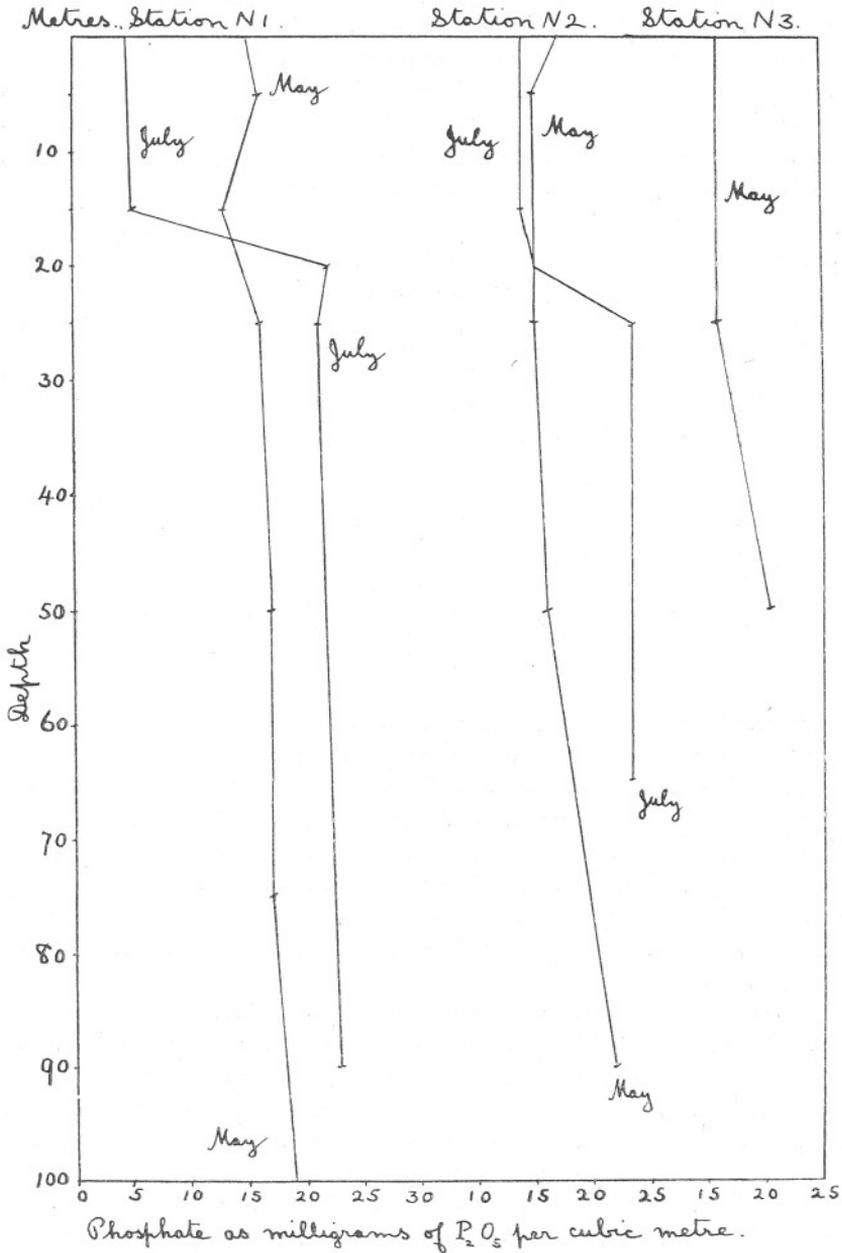


FIG 7.

## PHOSPHATE CONTENT OF THE NORTH SEA.

Since many of the determinations made by Raben were carried out upon water from the North Sea, it seemed to be of interest to examine samples from that region also in order to see whether values in better agreement with his results would be obtained. This was rendered possible through the courtesy of Dr. E. S. Russell, Director of the Fisheries Laboratory, Lowestoft, and of Mr. J. R. Lumby, who kindly collected the water samples. The analytical results are shown in Tables X and XI.

TABLE X.

Phosphate in North Sea, surface samples, April to July.

Date.	Position.	Lat. N.	Long.	Phosphate as mgrms. $P_2O_5$ } per cubic metre.
13/4	Cross Sand Lightship	—	—	35
„	Inner end of Stanford Channel, Lowestoft	—	—	40
„	10' E. × N. from Tyne	55° 4'	1° 8' W.	36
„	60' E. × N. from Hartlepool	55° 8'	0° 22' E.	36
3/5	Do.	55° 23'	1° 22' W.	15
11/7	Off Newbiggin Point	55° 15'	1° 20' W.	8
25/7	18' N.E. × E. Tyne	—	—	11

It may be seen from Table X that the values found are quite similar to those for the English Channel for the same months.

Table XI also gives figures quite in accord with those found off Plymouth, but far lower than Raben's values for North Sea water. It should be noted that the figures in Table XI are not as uniform as might be expected, as in several instances the surface values are slightly higher than the bottom. This may be connected with the circumstance that there was a delay of one month between the collection and analysis of these samples.

Of especial interest are the results for Stations 24 and 25 in the deeper water off the coast of Norway. The 280-metre sample is two and a half times as rich in phosphate as is the surface water; again, there is over twice as great a concentration of phosphate at the bottom at Station 25 as at Station 23, with a depth of 70 metres. Near a coast there is usually more vertical mixing of the water than there is at stations well out, such as E2 and N1, accordingly one may expect an abundant plankton where deep water approaches the land or a submerged bank which causes upwelling. The phosphate values found support the views put forward by Natterer in this connection.

TABLE XI.

Phosphate in North Sea, England to Norway, May 3rd to 6th, 1923.

Station.	Lat. N.	Long.	Depth in metres.	Phosphate as mgrms. $P_2O_5$ per cubic metre.	Notes.
1	54° 32'	0° 2' W.	0	21	Near Tyne.
1	"	"	60	19	
2	54° 39'	0° 11' E.	0	19	
2	"	"	65	15	
8	54° 54'	0° 34'	0	17	
8	"	"	70	23	
10	55° 23'	1° 22'	0	15	
10	"	"	55	17	
13	56° 8'	2° 35'	0	14	
13	"	"	70	16	
14	56° 26'	3° 0'	0	17	
14	"	"	65	17	
15	56° 38'	3° 24'	0	25	
15	"	"	60	19	
16	56° 31'	3° 37'	0	11	South of usual course.
16	"	"	60	16	
18	56° 45'	3° 36'	0	20	
18	"	"	50	17	
22	57° 0'	4° 5'	0	18	Course more northerly, heading to Udsire.
22	"	"	60	18	
23	57° 30'	4° 15'	0	14	
23	"	"	70	17	
24	57° 59'	4° 25'	0	14	
24	"	"	100	24	
25	58° 28'	4° 34'	0	14	
25	"	"	280	36	

## SEASONAL CHANGES IN THE PHOSPHATE CONTENT OF FRESH WATER.

The changes occurring in the sea are naturally not without a parallel in fresh water, the study of which shows how minute is the amount of

phosphate left unabsorbed by the plankton during the summer. The fresh waters available for study were as follows :—

Staddon reservoir.—This is a cement-walled tank  $22 \times 8$  metres and about 2 metres in depth. It receives surface drainage water in wet weather, and at all times it receives through an inlet pipe the overflow from a small spring, which may very well be contaminated as it issues out. This is situated at about 200 feet elevation on the east of Plymouth Sound, upon the Staddon Grits, a formation of the Lower Devonian. There are no trees surrounding it.

Maryfield quarry pond.—This has precipitous slaty sides and seems to depend upon rainfall for its water, though it may be replenished by a small spring below water level, and in very wet weather some surface water may find its way in. It is situated upon Middle Devonian Slates in the Antony district of Cornwall, about five miles east of Staddon and at an elevation of about 150 feet. It is surrounded by trees, which shade it to some extent. The dimensions are roughly  $80 \times 80$  metres, with a depth of 2 to 3 metres in the middle.

Plymouth tap is supplied from Burrator Reservoir on Dartmoor.

TABLE XII.

Seasonal changes in phosphate, expressed in milligrams of  $P_2O_5$  per cubic metre.

Date.	Staddon Reservoir.	Inlet.	Date.	Maryfield, quarry pond.	Date.	Plymouth, town tap.
19/8/22	32*	—	21/10/22	59*	—	—
2/10	91*	—	21/1/23	57*	27/1/23	19*
4/11	75*	—	18/2	42*	—	—
23/2/23	128*	119	30/3	14	31/3	3
3/4	3.5	—	15.4	5	—	—
16/4	2	82	24/4	0	—	—
1/5	6	112	6/5	0.5	1/5	3
30/5	0	81	2/6	3	5/6	0.5
15/6	11	78	24/6	3	—	—
23/6	19	60	—	—	—	—
29/6	15	86	30/6	3	5/7	5.5
26/7	9	116	26/7	3	30/7	1.5
24/8†	0	100	24/8	0	24/8	0

\* Stored till analysed early in April.

† A slight turbidity in all three samples rendered the tint impossible to match with exactness. They were taken after rain.

The phosphate analyses are shown in Table XII, and are plotted in Fig. 8. It might be thought that the phosphate values were largely influenced by dilution with rain water, but electrical conductivity measurements show that this is not the case. That for the Staddon inlet is quite usual for a calcareous water, the reservoir values are some-

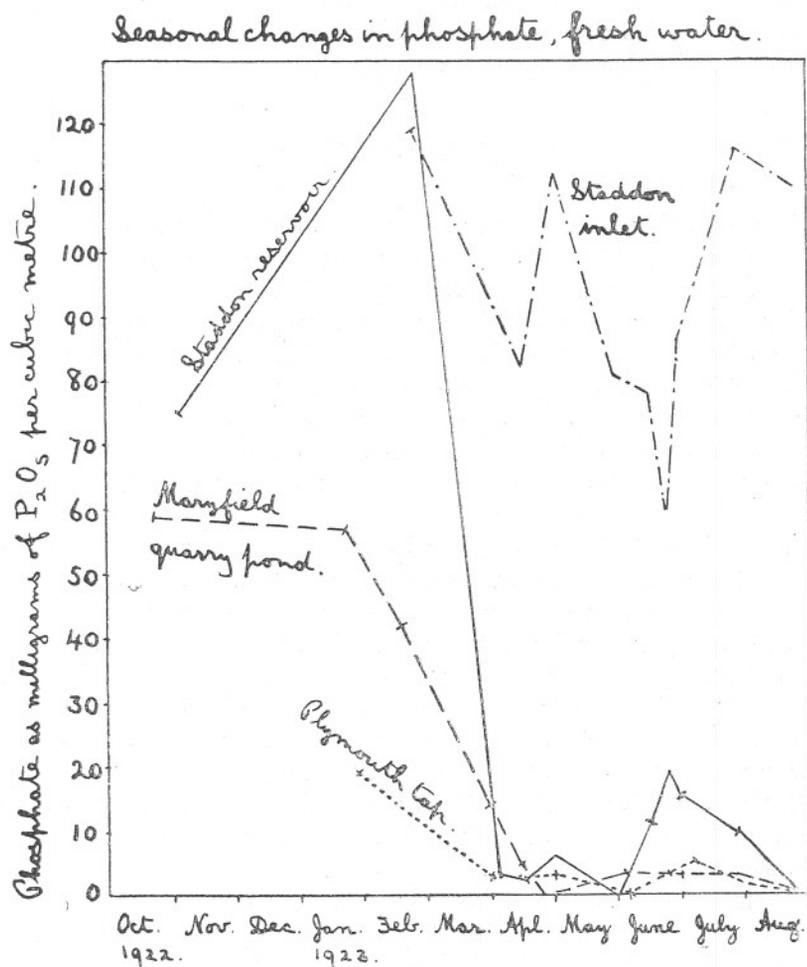


FIG. 8.

what lower: Maryfield quarry pond has, as a rule, a lower conductivity than has Staddon reservoir, and both are alkaline, around pH8 according to the season. Burrator water is at pH6.4-6.8, and its conductivity is only about one-tenth that of Staddon inlet. Though these waters are so different, two being quite "hard" and one very "soft," yet their changes in phosphate are closely similar, as may be seen in Fig. 8. It must be

pointed out, however, that the winter values given may be somewhat too high, since it is possible that phosphate was regenerated from plankton—more plentiful near the surface—during the period of storage. In general the Staddon reservoir has a much more abundant plankton during summer than has the Maryfield pond. In it the vernal outburst is followed by a period when but little algal life is found. The former becomes rich owing to its minute plankton and floating masses of *Spirogyra*, etc., absorbing the phosphate supplied by the inlet pipe.

#### APPROXIMATE ESTIMATION OF THE ALGAL PLANKTON CROP AND OF FISH PRODUCTION BASED ON PHOSPHATE CONTENT.

In the section dealing with the *Nitzschia* culture it was shown that the production of  $1 \times 10^9$  diatoms of the species grown consumed 1.12 mgrm. of phosphate reckoned as the pentoxide.

By measuring the areas between the ordinate and the March and July curves for phosphate at Station E1, as given in Fig. 5, it was ascertained that 2070 mgrm. of  $P_2O_5$  was consumed in the water column from 70 metres to the surface, having one square metre as its base. In other words, an average content of 37 mgrm. per cubic metre on March 7th had by July 10th fallen to 7.4 mgrm. In round numbers there was a consumption of 30 mgrm. per cubic metre or 2.1 gm. in the whole column. If the winter value be taken at 49 mgrm., the value found at L3, the consumption may be taken as 40 mgrm. in the same period.

Taking, however, the lower figure which was directly determined and the phosphate factor for diatom production, namely, 1.12 mgrm. per  $1 \times 10^9$  diatoms, it may be seen that each litre of water could produce  $26,800 \times 10^3$  diatoms of this species, provided nothing else grew in the water. Up to 30,000 diatoms per c.c. were found by the writer in a fresh water pond. These may be compared with the figures  $462 \times 10^3$  and  $464 \times 10^3$  given by Allen (1919) as the minimum values for plankton organisms per litre found early in August and September in sea water between Stations L2 and L3. The value found in the sea is only 1.7 per cent of that calculated from the phosphate consumption, because the algal plankton is eaten up by the smaller animal organisms, and serves indirectly as the food of all animal life in the sea.

Converting the above estimate per litre into per cubic metre it is seen that  $26.8 \times 10^9$  diatoms could be produced, or in the 70-metre column the enormous number  $188 \times 10^9$ . To be able to convert the numbers into weight it is necessary to either weigh diatoms directly or to know their phosphate content. An approximation to this may be obtained as follows: According to figures quoted by Czapek (1921) and Strasburger (1921) leaves may be taken as containing phosphate as pentoxide equiva-

lent to 15 per cent of their ash. Leaves were chosen as being assimilating organs, and so nearer to algæ than other parts such as wood or roots. Taking the ash as 10 per cent of the dry weight and the latter as 10 per cent of the moist weight, the phosphate content of the fresh plant is 0.15 per cent. Making the approximation 0.2 per cent  $P_2O_5$  as the phosphatæ content of diatoms, and using the value 2.1 grm.  $P_2O_5$  as the amount consumed in the whole column, it follows that 1.05 kilograms of diatoms could be produced; as a matter of fact if diatoms are not produced other unicellular algæ are, and their phosphate content must be very similar.

After this estimate was made, data relating to the phosphate content of algæ were found in the Fertilizer Resources of the U.S.A., pp. 225-9. Analyses by Barlow for three species of *Fucus* give 0.43 per cent phosphate on the dry weight. Determinations quoted from Tom show that *Fucus* has 24.2 per cent dry weight, which leads to the value 0.11 per cent phosphate on the wet weight. Tom's figure, 17.7 per cent for the dry weight of *Laminaria*, may be rounded off as 20 per cent, since there is a considerable variation; combining this with the value given by Russell, 0.66 per cent as a maximum for phosphate calculated on the dry weight the value for the wet weight works out at 0.13 per cent. Analyses made at the Connecticut State Experimental Station give as a mean for five algæ 0.14 per cent of phosphate as pentoxide, calculated on the wet weight. These figures, 0.11, 0.13, 0.14, show that the original estimate of 0.15 per cent  $P_2O_5$  as the phosphate content of unicellular algæ was probably fairly correct. Using it, instead of 0.2 per cent, the calculation of the algal plankton in the 70-metre column gives the result 1.4 kilogram, or  $1.4 \times 10^6$  per square kilometre.

When this result is compared with the value given as a minimum by a less exact method, the change in alkalinity of the water (Atkins, 1922), the agreement is extraordinarily close when a certain assumption is made, namely, that the carbohydrates of the algal cell, including protein carbon, calculated as a hexose sugar, amount to 15 per cent of the wet weight. This assumption was made as a consequence of Tom's figures for the total dry weight, and before the agreement was found by calculation. The alkalinity results gave an estimate of 1 kilogram per 4 square metres down to a depth of 83.3 metres. Converting this into the wet weight of algæ in a 70-metre column the value reached is 1.4 kilogram. The exact agreement is, of course, fortuitous in view of the assumptions; but it shows that the methods must have a certain degree of reliability, or rather it confirms the alkalinity result, for the phosphate method involves only one assumption, that the percentage of phosphate in the algal plankton is close to that of the larger brown algæ.

Turning now to the question of the phosphate content of marine invertebrates, according to Clarke and Wheeler (1922), only trifling quantities are, as a rule, found; certain analyses for calcareous algæ quoted by these authors are also very low, usually a trace to 0.00 per cent in the calcareous portions. The highest record is 0.18 per cent. The shells of crustacea are, however, fairly rich in phosphate, 4.07 to 6.70 per cent being the value for a medium-sized lobster, expressed as  $P_2O_5$ . Tricalcic phosphate is, indeed, the main constituent of the ash of the minute crustacea, as shown by Clarke and Salkover (1918), from which doubtless young fish obtain much of their phosphate. The shells, too, of certain brachiopods contain 75–90 per cent of tricalcic phosphate, and some worm tubes are notably phosphatic. The various amounts in the hard portions as well as in the softer tissues make it impossible to give even an approximation to the weight of invertebrates that could be produced each year.

With respect to fishes a greater uniformity is found. Atwater (1888) gives 0.514 per cent as the average value for the pentoxide of the flesh of fifty-five species. He quotes Sempolowski as giving the following figures for the phosphoric acid in the whole fish, wet weight:—

<i>Pleuronectes limanda</i>	.	.	.	.	1.25
<i>Gadus aeglefinus</i>	.	.	.	.	1.22
<i>Trigla gurnardus</i>	.	.	.	.	1.78
<i>Raia radiata</i>	.	.	.	.	0.91
<i>Acanthias vulgaris</i>	.	.	.	.	0.98
					—
Mean	.	.	.	.	1.27 per cent.
As $P_2O_5$	.	.	.	.	0.95 per cent.

It may be seen that the bony fishes are considerably richer in phosphate than are the cartilaginous. Seeing that they constitute by far the larger amount of fish in the sea one may take as an approximation 1 per cent of  $P_2O_5$  for fishes in general. Now if all the phosphate used up in the 70-metre column were converted into fish it could yield each year 210 grams of fish, or roughly 1 kilogram per 5 square metres. Since there are also vast numbers of plankton and bottom-dwelling animals this is, of course, a very large overestimate; the figure yields the value  $2 \times 10^5$  kilograms per square kilometre. In the absence of precise data one may perhaps assume that the fish represent between 1 per cent and 1 per thousand of this possible total quantity, which gives an estimate of between 200 and 2000 kilograms per square kilometre in water 70 metres in depth.

## METHODS FOR THE ANALYSIS OF PHOSPHATES.

Details of the usual methods where moderate quantities are involved may be found in the text-books; but their use for quantities of the order of one milligram or less per litre, reckoned as  $P_2O_5$ , involves the use of inconveniently large volumes of liquid. In precipitating with magnesia mixture the resulting ammonium magnesium phosphate is usually weighed after converting into pyrophosphate. Recently, however, Jones and Perkins (1923) have given details of a method in which the double salt may be weighed directly. Using the ammonium molybdate method of precipitation, Kleinmann (1919) has found that it is permissible to weigh as ammonium phosphomolybdate. The work of Posternak (1920) on the variability of this precipitate should, however, be remembered.

A very delicate reaction was developed by Pouget and Chouchak (1909, 1911) into a colorimetric or nephelometric means of estimating phosphates, using strychnine sulphate and sodium molybdate. The reagents produce a yellow opalescence. This has since been used by several workers, notably Kleinmann (1919), Embden (1921), who converted it into a gravimetric method, and by Matthews (1916-18). Embden found it convenient to use the resulting strychnine phosphomolybdate precipitate for work with solutions containing 1.0-4.0 mgrm.  $P_2O_5$ , since the precipitate is about thirty-nine times as heavy as the corresponding amount of pentoxide. The precipitation being performed in the cold renders this method specially suitable for the estimation of phosphate in the presence of organic phosphates, which are easily hydrolysed.

Matthews (1916-18) used the Pouget and Chouchak colorimetric method for estimating the phosphate in 500 c.c. of sea water after precipitation as ferric phosphate. The method was adopted after a very careful comparison with others available.

Raben (1916-20), working with Brandt (1916-20) at Kiel, precipitated the phosphate in 10 litres of filtered sea water by means of ferric chloride. After an elaborate purification the phosphate was determined gravimetrically as phosphomolybdate.

The results for sea water from various sources are from 51 mgrm.  $P_2O_5$  per cubic metre in May to 221 in November. It may be said that these values are greater than those obtained by Matthews, 0.06-0.01 or less, expressed in milligrams of  $P_2O_5$  per litre. Matthews also obtained evidence for the existence of a soluble compound of phosphorus, which can be converted into phosphoric acid by oxidising agents. The results obtained by the writer for phosphate in sea water are in complete agreement with those of Matthews, though obtained by an entirely different method. No explanation can as yet be offered as to why these differ so much from the very careful determinations of Raben and his co-workers.

A new method of great delicacy was developed by Denigès (1920, 1921), and was found by him to agree with the gravimetric method of Posternak (1920). The latter showed that the composition of the ammonium phosphomolybdate precipitate varies largely according to the proportions of the various salts present and to the temperature of precipitation; he accordingly worked out a process in which a barium phosphomolybdate of constant composition may be obtained.

#### METHOD OF DENIGÈS FOR PHOSPHATES.

Two reagents are required for the "cœruleomolybdic" method of Denigès: (a) 10 per cent ammonium molybdate and pure sulphuric acid in equal parts by volume, and (b) stannous chloride, freshly prepared from 0.1 grm. of tin dissolved in 2 c.c. of hydrochloric acid with one drop of 3-4 per cent copper sulphate and made up to 10 c.c. On mixing a few drops of (a) with 10 c.c. of the liquid to be tested and adding one or two drops of (b), an intense blue appears in the presence of phosphate. Denigès employed this reaction for the analysis of biological products, but it was used in a slightly different form by Florentin (1921) for the determination of the phosphate content of fresh waters. Denigès considers that the maximum delicacy of the method is for solutions containing 0.5-10 mgrm. of phosphorus as phosphoric acid.

Florentin has employed it for the estimation of phosphate equivalent to 0.01-5.0 mgrm. of  $P_2O_5$ . He makes up solution (a) with 100 c.c. of 10 per cent ammonium molybdate plus 300 c.c. of 50 per cent (by volume) sulphuric acid. For analysis 10 c.c. of water is taken, to which are added three or four drops of (a) and one drop of (b), or three drops of (b) if more than 2 mgrm. of  $P_2O_5$  is present. The blue colour developed reaches its maximum in less than ten minutes. Comparison is then made with standards containing known amounts of phosphate, or indigo carmine for greater permanency. The acidity prevents the production of blue with molybdate alone. According to Florentin more than 0.1 grm. per litre of  $Na_2SiO_3$  gives a colour. As shown in an accompanying paper by the author no such amount of silicate has been found in any of the natural waters examined, for which 0.006 grm. per litre  $SiO_2$  (or 0.012 grm. approximately of silicate) is a high value.  $H_3AsO_4$  gives a blue colour similar to that given by phosphate, so any traces present are included in the phosphate estimation.

The writer has made use of the reagents according to Florentin's formula for (a), and has found it advisable to use 100 c.c. of the water to be tested owing to the minute traces of phosphate present. To this quantity of fresh or sea water 2 c.c. of (a) are added and five drops of (b), and the blue tint is examined in a graduated 100 c.c. cylinder with

a tap near the base. The tint is compared with that given by a convenient strength of phosphate solution, usually one containing the equivalent of 0.05 mgrm. of  $P_2O_5$  per litre. The standard solution falls off somewhat on keeping; a 6 per cent decrease was observed in  $2\frac{1}{2}$  hours, by comparison with a fresh solution. This amounts to 1 per cent per half-hour approximately, so when examining a series a fresh standard is mixed after about half an hour. Taken over a twenty-five hour period, however, the decrease was only 1 per cent per hour. Sometimes the solutions quickly develop a turbidity. This trouble has been traced to the stannous solution, which is apt to give the precipitate if added to the sample before the acid molybdate, or if added in too great amount, or if heated for an undue length of time when being prepared. It was, moreover, noticed that the precipitate came more readily in distilled or naturally occurring fresh water than in salt water, in which the sodium chloride apparently lessens hydrolysis by diminishing the percentage ionised.

When adjusting the height of the stronger solution to match that of the lighter at the 100 c.c. level the columns are viewed standing on a thin glass shelf below which is opal glass. The sides and back of the stand are black. Accuracy is assisted by having on the opal glass a white card on which are ruled black lines. This is adjusted so that half of the field of each column is occupied by the card, and half by the opal glass. The tubes are screened in front by cardboard.

Before trying the cylinders, which are now used invariably, Nessler tubes containing 50 c.c. were used; a series from 0.05–0.01 mgrm.  $P_2O_5$  was made up, and it was found that the members could readily be arranged in the correct order. The use of the cylinders increases the accuracy, as it is usually possible to get duplicate readings to within 2 c.c. on the column. Good agreement may also be obtained against a standard of a different strength. Thus sea water tested against a 0.05 standard gave:—

$$\text{1st reading 66, viz. } \frac{0.05 \times 66}{100} = 0.0330 \text{ mgrm. } P_2O_5 \text{ per litre.}$$

$$\text{2nd reading 67.5} \quad = 0.0337 \quad \text{,,} \quad \text{,,} \quad \text{,,}$$

Against a 0.04 standard the reading was 82, corresponding to 0.0328 mgrm.  $P_2O_5$  per litre. The colour is not sufficiently intense with such dilutions to permit of the use of the Duboseq colorimeter, on account of the shorter length of liquid column available.

There is, however, one source of error which remains as yet quite unexplained. On standing with the reagents sea water and certain fresh water samples from ponds develop a slight yellowish tint. This is not noticeable as a rule till after five minutes, so the comparison should be made before it has time to develop, and as soon as the blue has reached its

maximum intensity. The colour is not given by the acid molybdate alone. An exact match may nevertheless be obtained even in the presence of the yellow tint by adding drops of very dilute Bismarck brown to the standard. The result got by trying to match the tints without the addition of the brown is usually about 0.004 mgrm. per litre too low.

It must be added that blank estimations are made from time to time by adding the reagents to distilled water. With freshly made up molybdate mixture no more than 0.0005 mgrm. per litre need be subtracted for the tint given by the reagents, 0.002 mgrm. is a very usual value for molybdate mixture stored in the dark, and after some time in the light as much as 0.004 mgrm. may have to be deducted.

It should be stated that the standard phosphate solutions were made up by diluting a solution of sodium ammonium hydrogen phosphate equivalent to 5 mgrm.  $P_2O_5$  per c.c. The stock solution was diluted to give 50 mgrm. per litre, and for general use this was further diluted to 0.5 mgrm. per litre. By taking 10 c.c. of this and making up to 100 c.c. the usual standard 0.05 mgrm.  $P_2O_5$  per litre was obtained. Solutions not conveniently matched against this strength were either diluted suitably or else a more concentrated standard was used. Such solutions are very liable to grow moulds or minute green algæ, which, of course, alter their phosphate content. The addition of a little toluene was, however, found to prevent this for some months at any rate.

It is also noteworthy that Florentin pointed out that the presence of the acid prevents the molybdate alone from giving a blue with stannous chloride. On one occasion through an error the acid molybdate solution was made up to contain only 25 per cent of sulphuric acid; as usual 2 c.c. of this was added to 100 c.c. sea water, followed by five drops of stannous chloride. The intense blue which developed appeared to denote an absurdly large phosphate content, and on repeating the estimation with fresh reagents the mistake was discovered and Florentin's observation was recalled to mind.

As previously mentioned it is possible to get readings in duplicate, when comparing the blue tints in the 100 c.c. cylinders, which agree to 2 c.c. This limit, using a 0.05 mgrm.  $P_2O_5$  per litre standard, corresponds to 0.001 mgrm. per litre. Even taking it that the reading may be 2 c.c. too high or too low, the error only becomes  $\pm 0.001$  mgrm. per litre. This should not be surpassed in clear solutions in which no yellow tint develops. With slightly turbid solutions or those which are tinted the error may, of course, be greater, though use of dilute Bismarck brown materially reduces it. Matthews, using Pouget and Chouchak's method on the phosphate from 500 c.c., considers that the estimation is accurate to about 0.003 mgrm. per litre. The method of Denigès, as used by the writer, gives results which are in most cases accurate to  $\pm 0.001$  mgrm.

per litre, and may certainly be considered at least to equal those obtained by the Pouget and Chouchak method in accuracy. Furthermore, since the method of Denigès requires only 100 c.c. the phosphate actually estimated is only one-tenth of the concentration in milligrams per litre.

Matthews found that, using filtered sea water, duplicate determinations required five hours. The filtration, moreover, took upwards of sixteen hours, and was necessary on account of the risk of contamination of the precipitate. Using the method of Denigès an estimation occupies ten minutes, and unless particles of phosphate are suspended in the liquid no error results from the presence of the ordinary amount of algal plankton. It must be concluded that this mode of estimation has many advantages.

It may be added that to convert the conventional  $P_2O_5$  values into the more rational values for the  $PO_4$  ion the factor 1.338 may be used to multiply the former. The factor is very approximately  $\frac{4}{3}$ . For the converse the factor 0.7474 should be used, which may be taken as  $\frac{3}{4}$ .

#### SUMMARY.

1. The phosphate content of uncontaminated streams and fresh water supplies examined was under 0.05 parts per million reckoned as  $P_2O_5$ . To convert to  $PO_4$  the factor 1.338, very approximately  $\frac{4}{3}$ , may be used.

2. A pure culture of *Nitzschia closterium* W. Sm., in sea water enriched with Miquel's solution, multiplied in numbers up to over three million per cubic centimetre, when the phosphate was all used up. It was ascertained that 1.12 mgrm., expressed as  $P_2O_5$ , is required for the production of  $1 \times 10^9$  diatoms during the early stage of the culture. One gram of the pentoxide suffices for  $9 \times 10^{11}$  diatoms.

3. Sea water insolated in the Laboratory decreases rapidly in phosphate till none is left. Samples taken in winter show a less rapid decrease than those taken in spring. This is due to their smaller content of algal plankton. Ferric chloride removes phosphate from sea water or culture solutions very completely.

4. The phosphate content of sea water falls from a value of 0.036 mgrm. per litre at the surface at Station E1 in March to zero in July. The bottom value also falls to 0.011 mgrm. in July, so that there is a consumption throughout the column of water to 70 metres of 0.030 mgrm. per litre. Similar changes take place in Plymouth Sound and at the Hydrographic Stations E2, E3, and N1-N3. The surface water is almost free of phosphates from May to August.

5. A few determinations made indicate the same seasonal change in the North Sea. The deep water off the Norwegian coast acts as a reservoir of phosphate, which presumably gets depleted during summer; 0.036 mgrm. per litre was found there on May 6th at 280 metres. The North Sea values for phosphate are much lower than those found by Raben, and the phosphate analyses in general agree well with the results obtained by Matthews. As regards the seasonal change the results are in agreement with both workers.

6. The phosphate of fresh water ponds was found to fall almost to zero early in April, and to continue low throughout summer.

7. An estimate may be made of the total algal plankton crop each year, using the figures recorded in §2 and §4 of this Summary. Since 1.12 mgrm. of  $P_2O_5$  suffices for  $1 \times 10^9$  diatoms, each litre of sea water could produce 26.8 million diatoms for a consumption of 0.030 mgrm. As many as 30 million diatoms per litre were found by the writer in a fresh water pond, so these large figures, as calculated, need not seem impossible.

Taking it that each cubic metre to a depth of 70 metres loses 30 milligrams of phosphate as  $P_2O_5$  and that the phosphate content of the algal plankton is 0.15 per cent, calculated on the wet weight, it results that the column of water produces 1.4 kilograms algal plankton per square metre of sea. If one assumes that the carbon content of the algæ, reckoned as a hexose sugar, amounts to 15 per cent of the wet weight the calculation made by the writer (1922) from the seasonal change in alkalinity gives an identical value 1.4 kilograms. The exact agreement is fortuitous, but it lends support to the validity of the alkalimetry method.

8. The colorimetric method of Denigès was found very convenient for the analysis of waters containing 0.050 to 0.001 mgrm. of  $P_2O_5$  per litre. An accuracy of  $\pm 0.001$  mgrm. can be obtained in clear solutions free from tint, and results to within  $\pm 0.002$  may readily be obtained. For samples which develop a yellowish tint with the reagents it is convenient to add a little Bismarck brown to the standard.

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NOTE.—Up to the end of November, 1923, the phosphate content of the fresh waters studied has been far below the 1922 values, obtained on stored samples. This indicates that the possible error from storage, mentioned on p. 140, l. 1-3, may be very considerable. The accuracy of Fig. 8 is thus impaired.