

Nitrate in the Sea.

By

H. W. Harvey, M.A.

Hydrographer at the Plymouth Laboratory.

With 3 Figures in the Text.

IN view of the need of nitrates for the growth of vegetable plankton, it was desired to evolve a more or less rapid method of estimating the minute trace of nitrate which occurs in sea-water, preferably one which would have its greatest accuracy in dealing with waters almost entirely depleted of nitrate. The existing methods were lengthy and subject to a systematic error which lowered their value, if it did not render them useless, in comparing the nitrate content of such almost entirely depleted waters.

Denigès (2) found that an acid solution of strychnine reduced by warming with amalgamated zinc produced a compound which gives a bright red colour with nitrites, and that a similar colour was given with nitrates on the addition of strong sulphuric acid. Kolthoff (3) examined this reaction and found that the colour was given with chlorates, chromates and ferricyanides, as well as by nitrites, with or without strong sulphuric acid, and by ferric iron as well as by nitrates with strong sulphuric acid. He noted further that more consistent results were obtained by using amalgamated zinc, than by zinc or magnesium as had been suggested by Scales and Harrison.

The colour fades on exposure to strong light.

Preliminary experiments showed that this qualitative "test" for nitrates was the most delicate of many examined and that under particular conditions it was capable of giving quantitative results.

NITRITES.

In a number of water samples from the English Channel, examined either on board ship or immediately after landing, no definitely detectable traces of nitrites could be found either by the Greiss-Ilosvay reagent or with the strychnine reagent without sulphuric acid, except in the case of inshore water subject to pollution. After storage an appreciable quantity of nitrite has been detected in several instances. In water from 830 metres in the Bay of Biscay, preserved with mercuric chloride when collected, and in water at various depths up to 3000 metres, similarly preserved, collected by the R.R.S. *Discovery* some 200 miles west of Portugal, no nitrite could be definitely detected.

IRON.

Sea-water has been repeatedly found to contain iron in solution. No trace of ferric iron could be detected in a number of samples of sea-water by adding either sulphocyanide or ferrocyanide. Nor could ferric iron be detected after addition to unfiltered sea-water of iron free hydrogen peroxide and acid, by which treatment ferrous iron is converted into ferric. It is only after the water, whether unfiltered or filtered, has been subjected to strong oxidation, such as by boiling with acid bromine water (4) or nitric acid (5) that a reaction for ferric iron is given. There is evidence that the iron in sea-water is present in organic compounds (5).

THE REAGENT.

When a solution of about 0.5 gram of strychnine sulphate in 25 c.c. of water is warmed with 2 c.c. of hydrochloric acid and 4 square inches of zinc foil, previously wetted with acid mercuric chloride solution to form amalgam on the surface, the less soluble "reduced strychnine" separates out. On continued heating for two or three hours on a water-bath, with the addition of more water from time to time to keep the volume between 10 and 25 c.c. and the addition of a few drops of sulphuric acid towards the end, an active compound is formed. The operation should be carried out away from the gases given off by a naked flame. The yield and time taken is somewhat capricious. By adding a drop of the liquid to a very dilute solution of nitrite, the reaction may be stopped when a satisfactory yield of the active compound has been obtained. After removing the zinc, about 300 c.c. of strong sulphuric acid are added. The reagent so prepared gives a red colour on addition to an equal volume of very dilute nitrate solution; the colour develops more rapidly after the reagent has been stored for several days.

It is essential that pure chemicals, free from nitrate and iron be used, otherwise the reagent gives a pink colour on addition to an equal volume of distilled water, due to the contained impurities. The "pure" zinc foil and "nitrogen free" sulphuric acid supplied by Messrs. British Drug Houses proved satisfactory.

THE ESTIMATION.

The depth of colour produced, that is, the amount of red substance, is dependent upon (a) the amount of active compound in the particular batch of reagent employed, and (b) the nitrate (or nitrite) in the sea-water. Equivalent quantities of nitrate and of nitrite give the same, or very nearly the same, depth of colour.

The fact that water from the English Channel in August gave no perceptible colour with the reagent, the nitrate having been completely utilised by diatoms, etc. (p. 82), is strong indirect evidence that the

colour developed with the reagent and sea-water is due to nitrate alone, except in the rather rare instances where nitrite also occurs.

The production of colour by the reagent with ferric iron is not of the same order of delicacy as with nitrates. In an experiment where the addition of 50 milligrams of nitrate-nitrogen per cubic metre to a sea-water, almost depleted of nitrate, gave rise to a decided red coloration on addition of the reagent, no increase in colour could be discerned due to the addition of an equal quantity of ferric iron to the sea-water. The addition of 5000 mg. per cubic metre of ferric iron gave rise to a coloration considerably less than that produced by 50 mg. of nitrate-nitrogen. This evidence, together with the absence of detectable ferric iron in sea-water shows that the estimation of nitrate by this method is not likely to be materially affected by the presence of iron in the sea.

With a strong batch of reagent, that is, one containing a large amount of the active compound, a decided pink colour is given with a sea-water containing 10 milligrams per cubic metre of nitrate-nitrogen, and as little as 2 milligrams per cubic metre gives a detectable tint.

Up to a certain concentration of nitrate, the depth of colour, as measured with a Duboscq colorimeter or pair of glass cylinders, is directly proportional to the nitrate-content of the sea-water.

In an experiment with a sample of water, particularly free from nitrate, collected well off shore in the summer, the following proportional relations were found between the depth of colour given by samples of the water to which 10, 20, and 30 milligrams of nitrate-N₂ had been added per cubic metre.

(a)	(b)	Ratio of colour given by $\frac{(a)}{(b)}$
Sea-water alone	Sea-water +10 mg. nitrate N ₂ per m ³ .	$\frac{1}{4.75}$
Sea-water +10 mg. nitrate-N ₂ per m ³ .	Sea-water +20 mg. nitrate-N ₂ per m ³ .	$\frac{1}{1.9}$
Sea-water +20 mg. nitrate-N ₂ per m ³ .	Sea-water +30 mg. nitrate-N ₂ per m ³ .	$\frac{1}{1.44}$

By giving the depth of colour produced by the sea-water, to which 20 mg. of nitrate-N₂ per cubic metre had been added, the arbitrary value of 100 units of colour

Sea-water alone	gave 11 units.	$\left. \begin{array}{l} \\ \\ \\ \end{array} \right\} \text{Difference.}$
,, +10 mg. nitrate-N ₂ ,,	52.5	
,, +20 ,, ,, ,,	100	
,, +30 ,, ,, ,,	144	

Mean number of units of colour produced by the addition of 1.0 mg. of nitrate-N₂ per cubic metre = 4.4 units.

Hence the sea-water without any addition of nitrate, which gave 11 units of colour contained between 2 and 3 milligrams of nitrate-N₂ per cubic metre.

Above a certain concentration of nitrate, which varies with the batch of reagent employed, the colour falls off for each successive increment of nitrate until an orange and finally a yellow colour is produced.

In an experiment the following proportional relations were observed between the depth of colour given by a sample of sea-water to which varying quantities of nitrate had been added.

(a)	(b)	Ratio of depth of colour produced by (a) to that produced by (b).
Sea-water only	Sea-water + 20 mg. of added nitrate-N ₂	$\frac{1}{2.28}$ *
Sea-water + 20 mg. of added nitrate-N ₂	Sea-water + 40 mg.	$\frac{1}{1.29}$
Sea-water + 40 mg.	„ + 80 mg.	$\frac{1}{1.69}$
„ + 80 mg.	„ + 120 mg.	$\frac{1}{1.35}$
„ + 120 mg.	„ + 160 mg.	$\frac{1}{1.18}$
„ + 160 mg.	„ + 200 mg.	$\frac{1}{1.11}$
„ + 200 mg.	„ + 260 mg.	$\frac{1}{1.15}$

Assigning the value of 100 arbitrary units to the depth of colour developed by the sample to which 80 mg. of nitrate-N₂ had been added, then

Sea-water only gives		20 units of colour.
„ + 20 mg. of added nitrate-N ₂ per cubic metre gives		46 „ „
„ + 40 „ „		59 „ „
„ + 80 „ „		100 „ „
„ + 120 „ „		135 „ „
„ + 160 „ „		159 „ „
„ + 200 „ „		176 „ „
„ + 260 „ „		183 „ „

* Values shown are the mean of several colorimeter readings.

Plotting the units of colour against the nitrate added, Fig. 1 was obtained. By extrapolation the sea-water alone without added nitrate is shown to contain ca. 22 mg. of nitrate-N₂ per cubic metre. Above a total content of about 140 mg. per cubic metre the linear relation with the depth of colour ceases.

It was found that the colour developed by a definite quantity of nitrate dissolved in distilled water is usually less than when it is dissolved in sea-water, which apparently contains some substance which activates the reaction. Differences in salinity such as ordinarily occur have little or no effect on the colour developed; this is shown by plotting

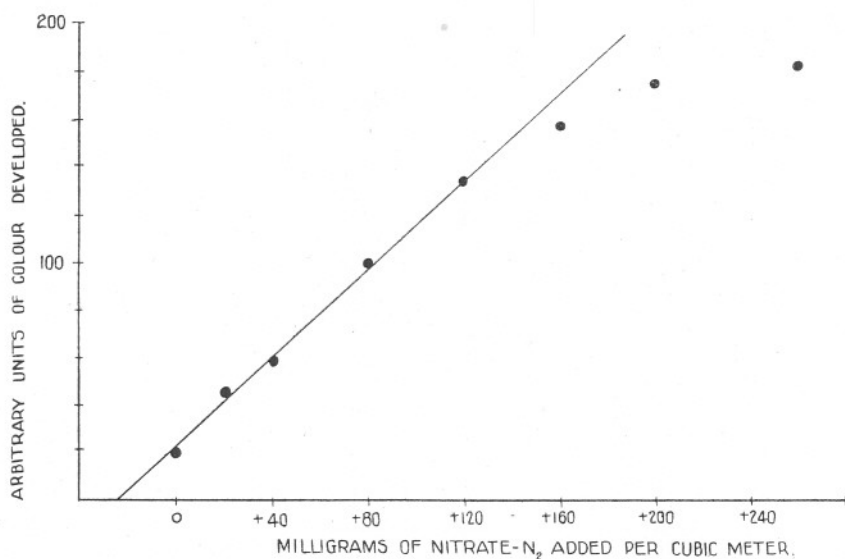


FIG. 1.

the units of colour developed by a sea-water at various dilutions (Fig. 2)

From a number of experiments it appears that the quantity of coloured substance produced due to the addition of the same quantity of nitrate to different sea-waters is very nearly the same, provided, of course, that the batch of reagent used and the conditions under which the reaction proceeds are the same. However, one striking instance has been found where the colour developed due to added nitrate was markedly less than with water from the same position but at lesser depths. This occurred in water collected twenty-two miles south-west of Plymouth on November 11th, 1925, at a depth of 70 metres.

Hence the comparison *inter se* of the depths of colour given by a number of sea-waters does not give the absolute relation of their nitrate contents,

although it does give a very fair approximation. To obtain the absolute relation it is necessary to add a known amount of nitrate to each sample and from the proportional increase in colour developed with the reagent to calculate the nitrate content of the sample. Thus if the colour developed by the sample of sea-water alone is taken as 100 arbitrary units, and after the addition of 50 mg. of nitrate-N₂ per cubic metre it is 170 units, it follows that 50 mg. per cubic metre give rise to 70 units of colour, and the sample contained $100 \times \frac{50}{70}$ mg. per cubic metre.

Where the sample contains much nitrate so that the depth of colour and the nitrate content are outside the range of the linear relation it is

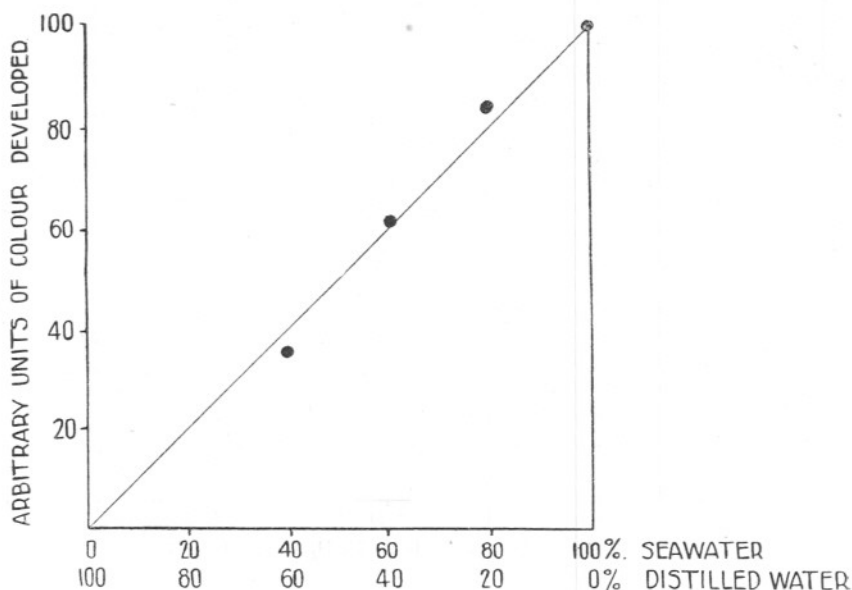


FIG. 2.

necessary to dilute the sample either with distilled water or with sea-water of low and known nitrate content.

Regarding the accuracy of the determinations, it must be borne in mind that 1 mg. per cubic metre is one part in 1000 millions, so a minute speck of nitrate-containing dust or other contamination will lead to a high value. For this reason duplicate determinations and the discarding of any "high" values due to chance contamination are necessary.

From these considerations, the method yields values of the nitrate content of clean water from the open sea which are a very close approximation to the actual content, provided the increment of colour due to the addition of a known amount of nitrate is determined for each sample of sea-water.

With waters polluted with dissolved organic matter in quantity, the method does not give quantitative results, since part of the nitric acid set free nitrates the organic matter. Thus the addition of 170 grams of glucose per cubic metre to a sea-water reduced the nitrate- N_2 found by 38%. The presence of much plankton in the sample will therefore affect the results. Hence with estuarine and polluted waters containing organic matter in solution—and in suspension—the values of nitrate content obtained are likely to be somewhat on the low side.

The determinations were mostly carried out by adding 10 c.c. of the reagent to 10 c.c. of sea-water in a stoppered test tube and making the colour comparison with a Duboscq colorimeter by the light of a "Fullo-lite" lamp, after the tubes had stood together for the same period in the dark, generally overnight, in order that the colour might fully develop and a fine precipitate of calcium sulphate, etc., might settle.

DISTRIBUTION IN THE SEA.

In 1912 Gran (6) wrote: "The occurrence of dissolved nutritive matter for algæ is on the whole insufficiently known, so we are compelled to estimate its importance by studying the distribution of the water masses, which may be conceived to carry nutritive substances out from the coastal layer or up from the deep water. . . . Where a deep going current is pressed against the coast banks, as off the west coast of Scotland or at the Faero Bank, eddying movements, not only horizontal, but also vertical will necessarily arise, and the water masses from the depths will be carried up to the surface. Nathansohn pointed out that ascending currents may produce a rich phytoplankton."

The only method of estimating nitrates in sea-water up to the present has been to reduce them to ammonia with nascent hydrogen. This method has been employed by Brandt and Raben (15). They found that the deep water of the Atlantic contained more than the surface layers, and that surface water in the North Sea contained less nitrate during the summer than in winter. It appears that the values obtained by this method, for the upper layers at all events, are excessive, being in the order of 60 to 100 milligrams of nitrate-nitrogen per cubic metre. The results of these observers did not suggest that deficiency of nitrate was a limiting factor for plant growth in the sea.

The results obtained in 1925, however, by the method here described, indicate that lack of nitrate does limit plant growth.

DISTRIBUTION OF NITRATES DURING 1925.

With one exception, a number of samples from the surface water well away from land or submarine ridges contained a small amount of nitrate (Tables I, II, III, V).

The deep water, on the other hand, was particularly rich in nitrate (Tables III, IV, V).

As land was approached on the south coast of Ireland the upper layers were found to be rich in nitrates in June (Table VI). They abounded in animal and vegetable life.

In the Faero Shetland Channel, where the Atlantic Stream runs into the Norwegian Sea, both deep water and the upper layers are rich in nitrate. In this area and off the south-west of Ireland it is presumed that considerable vertical mixing takes place between the surface and water below about 40 metres during the winter and early summer months. Later in the summer warming of the surface layers is likely to restrain vertical circulation, when a greater difference in the nitrate content of the upper stratum and the water below may be expected. In contrast with the water to the westward of Portugal (Table IV) in these higher latitudes light will penetrate to a lesser depth, and the greater part of the photosynthesis by plants is likely to occur in the upper 30 or 40 metres, as was clearly the case in early summer off Norway (Table V).

TABLE I.

Surface samples collected en route between South America and Europe, and kept in darkness until analysed on September 9th.

Date collected.	Temp. °C.	Lat.	Long.	Nitrate-N ₂ mg. per cubic metre.
August 13, 1925 . . .	27.8	0°20'S.	47°16'W.	8
August 16	27.2	9°25'N.	38°48'W.	5
August 18	24.2	19°10'N.	30°15'W.	5
August 21	22.2	28°12'N.	21°20'W.	5
August 24	19.4	37°54'N.	10°16'W.	5
August 26	16.4	48°27'N.	7°10'W.	5

TABLE II.

Surface samples collected en route between Britain and Canada, preserved from bacterial action by mercuric chloride.

Date collected.	Temp. °C.	Salinity parts per thousand.	Lat.	Long.	Nitrate-N ₂ mg. per cubic metre.
August 27, 1925 (in Labrador Current)	10.0°	30	51°48'N.	55°00'W.	2
August 27	10.6	35	52°20'N.	50°00'W.	2
August 26	12.8	35	53°00'N.	40°00'W.	7
August 25	14.4	35	53°10'N.	30°00'W.	84

TABLE III.

Bay of Biscay. Lat. 45°50'N., Long. 9°00'W. Collected June 8th, 1925, by H.M.S. *Tomahawk*, preserved with mercuric chloride.

Depth.	Nitrate-N ₂ per cubic metre.
360 metres	160 mg.
830 „	ca. 200 „

TABLE IV.

Samples collected by R.R.S. *Discovery*. Preserved against bacterial action by the addition of mercuric chloride.

October 12th, 1925. Lat. 37°44'N., Long. 13°21'W.

Depth in metres.	Temp. °C.	Nitrate-N ₂ mg. per cubic metre.
0	21.10	15 ; 11
10	21.10	7
20	21.00	6
30	21.00	—
40	21.00	16
50	20.01	ca. 6
75	17.31	6
100	15.10	55
150	15.06	65
200	13.86	100
300	12.25	178 ; 158
500	10.94	200
1000	9.55	264 ; 262 ; 274
2000	4.81	ca. 265
3000*	3.10	ca. 265

TABLE V.

Samples collected by the S.S. *George Bligh* south-west of Norway, May 16th, 1925. Lat. 57°57'N., Long. 6°45'E. Kept in darkness until analysed on May 25th.

Depth	Nitrate-N ₂ mg. per cubic metre.
0 metres.	6, 4
20	16
40	77
100	138, 128
300	160

* Oct. 16th, 1925, Lat. 29°59'N., Long. 15°03'W.

TABLE VI.

Samples collected off the south coast of Ireland, kept in darkness until analysed on June 6th.

Collected May 14th, 1925. Lat. $50^{\circ}34'N.$, Long. $11^{\circ}17'W.$ in 1040 metres.

Depth.	Nitrate-N ₂ mg. per cubic metre.
0 metres.	170 mg.
462	190
1010	190

Collected May 12th, 1925. Lat. $49^{\circ}21'N.$, Long. $8^{\circ}00'W.$ in 138 metres.

Depth.	Nitrate-N ₂ mg. per cubic metre.
0 metres.	60 mg.
60	130
133	130

TABLE VII.

Samples collected by the s.s. *Explorer* in July, and kept in darkness until analysed.

Collected in Channel between Faeros and Shetland. Lat. $61^{\circ}27'N.$, Long. $4^{\circ}23'W.$, July 6th, 1925.

Depth.	Nitrate-N ₂ mg. per cubic metre.
10 metres.	67 mg.
60	160
300	160
900	160
1000	160

Lat. $61^{\circ}02'N.$, Long. $3^{\circ}22'W.$, July 6th, 1925.

Depth.	Nitrate-N ₂ mg. per cubic metre.
10 metres.	18 mg.
40	78
100	92
500	106
800	115

North-west of Faero Islands. Lat. $62^{\circ}53'N.$, Long. $9^{\circ}05'W.$, July 4th, 1925.

Depth.	Nitrate-N ₂ mg. per cubic metre.
0 metres.	85 mg.
10	70
30	115
80	over 200
100	" "
300	" "
495	" "

IN THE ENGLISH CHANNEL.

A growth of diatoms had become apparent after the winter cessation, previous to the analyses being commenced in April, 1925. On April 22nd it is seen from Table VIII that the water at Station E1, twenty-two miles south-west of Plymouth on the line Plymouth-Ushant, was depleted of nitrates in the upper layers. By May 13th the depletion was more marked in the deep water, while farther to the southward at Stations E2 and E3 in water of slightly higher salinity and greater depth the supply of nitrate was considerable.

TABLE VIII.

Nitrate content in milligrams of nitrate-N₂ per cubic metre at various depths (in metres, m.) of water in the English Channel on the line Plymouth-Ushant.

	In Plymouth Sound, Station L ₁	Off west end of Plymouth Breakwater Station L ₂	5 miles S 21° W (true) from Plymouth Hoe Station L ₃	9 miles S 21° W (true) from Plymouth Hoe Station L ₄	22 miles S 21° W (true) from Plymouth Hoe Station E ₁	59½ miles S 21° W (true) from Plymouth Hoe Station E ₂	115½ miles S 21° W (true) from Plymouth Hoe Station E ₃
April 22nd, 1925	—	—	—	—	0 m. 4 5 m. 2 10 m. 2 25 m. 3 50 m. 24 67 m. 24	—	—
May 13th, 1925	—	0 m. 14	—	0 m. 2 40 m. 3	1 m. 2 10 m. 3 20 m. 5 30 m. 2 50 m. 9 69 m. 8	— 10 m. 55 — 30 m. 53 50 m. 15 92 m. 29	1 m. 67 10 m. 85 20 m. 85 30 m. 67 50 m. 67 — 105 m. 67
June 3rd, 1925	—	—	—	—	1 m. 3 10 m. 3 — — 40 m. 8 69 m. 9	—	—
July 8th, 1925	—	—	—	0 m. 4 40 m. 8	1 m. 4 20 m. 4 40 m. 4 70 m. 4	5 m. 3 25 m. 24 — 50 m. 29 98 m. 29	5 m. 40 — 25 m. 40 — 50 m. 40 — 167 m. 40

TABLE VIII.—*continued.*

	In Plymouth Sound Station L ₁	Off west end of Plymouth Breakwater Station L ₂	5 miles S 21° W (true) from Plymouth Hoe Station L ₃	9 miles S 21° W (true) from Plymouth Hoe Station L ₄	22 miles S 21° W (true) from Plymouth Hoe Station E ₁	59½ miles S 21° W (true) from Plymouth Hoe. Station E ₂	115½ miles S 21° W (true) from Plymouth Hoe Station E ₃
Aug. 5th, 1925	— — — — —	— — — — —	— — — — —	— — — — —	10 m. 0 20 m. 3 — 40 m. 3 69 m. 0	— — — — —	— — — — —
Aug. 31st, 1925	3 m. 9 — — — —	— — — — —	— — — — —	0 m. 0 — 40 m. 7 — —	5 m. 0 — 25 m. 9 — 50 m. 14 69 m. 14	— — — — —	— — — — —
Oct. 1st, 1925	0 m. 112 — — — — — —	0 m. 79 — — — — — —	0 m. 37 — — — — — —	0 m. 27 — — — 40 m. 27 — —	0 m. 6 10 m. 6 20 m. 21 30 m. 32 — 50 m. 32 66 m. (19)	— — — — — — —	— — — — — — —
Nov. 11th, 1925	0 m. 176 — — — — — —	0 m. 380 — — — — — —	— — — — — — —	0 m. 76 — — — 40 m. 43 — —	0 m. 68, 10 m. 68 20 m. 65 30 m. 56, — 50 m. 70, 70 m. (25, 30)	66 — — 41 — 69 —	— — — — — — —
Dec. 11th & 15th 1925	— — — — — —	0 m. 120 — — — — —	0 m. 110 — — — — —	0 m. 74 — — 40 m. 50 — —	0 m. 38 10 m. 40 25 m. 43 50 m. 48 70 m. 43 —	— 10 m. 106 25 m. ca120 50 m. ca120 — 90 m. 132	— — — — — —

On this date the water at these stations was still practically isothermal from top to bottom—characteristic of winter conditions—hence vertical mixing by wave motion and tidal streams over an uneven bottom at E3 was not restrained by differences in density.

In July the utilisation of nitrates by diatoms had caused a decrease at Stations E2 and E3. At E2 the decrease was most marked in the upper layers. In the interval between May 13th and July 8th, an increase in salinity had taken place at these two stations, probably due to a movement of more saline Atlantic ocean water into the area.

By August 5th diatoms had utilised all available nitrate at Station E1, and their dying out and lessened activity allowed a re-formation of nitrate in the bottom strata to become apparent by the 31st. During

this period, April to the end of August, the salinity of the water at E1 had remained practically the same, and there is no evidence of any marked movement of the water masses in the area. The rather scanty data indicate a low nitrate content of the inshore water during this period.

It is remarkable that a little nitrate remains in the surface layers of the tropical open ocean (Tables I and IV) where phytoplankton is scanty, while it is able to utilise practically all the nitrate in the English Channel. However, in the Channel, there are plenty of nutrient salts at the end of winter, and with the onset of sunny weather a very dense diatom

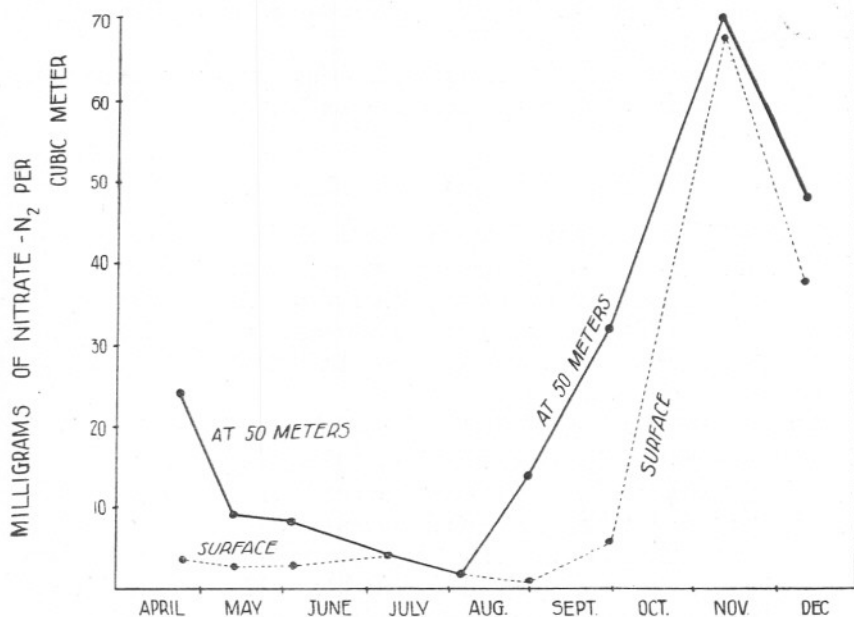


FIG. 3.—Nitrate content of water at Station E1, 22 miles south-west of Plymouth, during 1925 in the surface 5 metres and at 50 metres.

population springs up, gradually to die down during the summer months as the available nutrient salts diminish. In the surface water of the tropical and subtropical ocean there is a sufficiency of light all the year round, but plant growth is limited throughout the year by lack of nutrient salts. There is never a sufficiency to allow a dense vigorous growth to spring up, such as in the English Channel utilises the last traces of nitrate. In the latter area there must also be a greater *continuous* supply of phosphate and nitrate from the denser animal population.

From the end of August onwards the regeneration of nitrate is more rapid than its utilisation by diatoms. The burst of spring growth has died down sometime previously and decaying corpses become numerous.

The utilisation of nitrate in the upper 15 metres by the usual autumn flowering of diatoms is apparent on October 1st.

Between November 11th and December 11th a fall in nitrate occurred at Station E1, and I am indebted to Dr. W. R. G. Atkins for the observation that a fall also occurred in the phosphate, which had started to regenerate more rapidly than it was being utilised by August 5th—earlier than the nitrate. It is noteworthy that there was considerable sunshine during this period, November 11th to December 12th (average 3.6 hours per day).

Reference must be made to the low content of nitrate in the water at 70 metres at E1 on October 1st and November 11th. No reason is suggested for this.

Fig. 3 shows the seasonal variation in nitrate content of the upper 5 metres and of the 50-metre layer at Station E1.

THE REGENERATION OF NITRATE.

In 1910 Thomsen found nitrate-forming bacteria in bottom deposits from Kiel Fjord, derived from land washings, but not in sea-water or plankton (7). Lipman was unable to find nitrate forming bacteria in water from near Tortugas, but quotes a statement by Issatchenko that he had found nitrifying bacteria in the Arctic in 1908, but was unable to verify their presence later (8).

Surface water collected at Station E1 on August 5th to which had been added 200 milligrams per cubic metre of ammonium-nitrogen, was kept in the dark for six days at room temperature. At the end of this period no colour developed on adding the reagent. Water from a depth of 69 metres collected on the same day and treated in the same manner was kept in the dark together with a control to which mercuric chloride had been added. After six days a definite colour was obtained with the reagent, while no colour was given by the control.

The depth of colour indicated the formation of nitrate to an amount in the order of 7 milligrams per cubic metre. No nitrite was detectable. On repeating the experiment and keeping for eight days, likewise at room temperature, nitrate was again found in the water, but not in the control, and the amount formed was of the same order.

A further experiment showed that the addition of detritus collected about four miles off shore caused the formation of nitrate to take place in surface water to which a trace of ammonium salt had been added, whereas without the detritus no nitrate was formed. The controls in which bacterial action was stopped by mercuric chloride showed no development of nitrate.

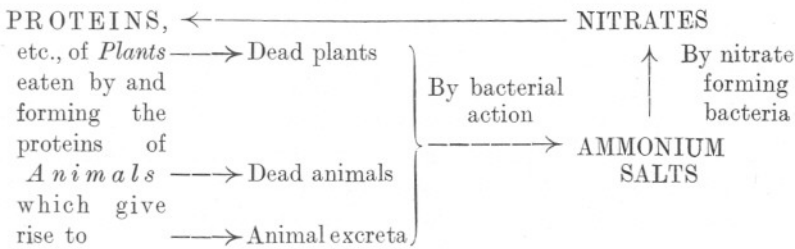
THE NITROGEN CYCLE.

Denitrifying bacteria have been found in water near the shore and in mud of the Baltic (9), but as pointed out by Gran (10) they are not likely to play a part in the economy of the open oceans, since the water almost invariably contains a sufficiency of oxygen for these bacteria, without their having recourse to attack the small quantities of nitrates present.

Azotobacter, fixing dissolved nitrogen, has been found in the slime of Baltic algæ (11) and in bottom deposits from near the land (12). There is no evidence as yet that they are general in occurrence or that they add more than a minute fraction to the combined nitrogen in the sea.

As far as we know the inorganic salts necessary for plant life are always present in sea-water in ample amount except phosphates (16) and nitrates. Iron is a possible exception. The nitrates are converted by the algæ into proteins, etc. Some of these algæ die, and from the decay of their corpses ammonium salts are produced. Others nourish marine animals which in turn are fed upon by other animals. These excrete the products of their metabolism and in due course die; ammonium salts are produced from their corpses and excreta by bacterial action.

Thus we have in the sea a closed cycle.



Phosphate is regenerated in a similar manner, and the evidence so far obtained shows that in both cases the cycle is practically a closed one, the increment due to land washings not being very great compared with the amount continually being regenerated from dead marine organisms.

A deficiency of nutrient salts limits both the rate of multiplication of vegetable plankton, and in all probability the rate of carbon assimilation as well (1). All the energy expended in the vital processes of plants and animals in the sea is derived from the energy of light absorbed during the course of carbon assimilation by phytoplankton—the fixed algæ being but a small proportion of the plant life.

Hence a quantitative knowledge of those factors, which control and limit the multiplication of vegetable plankton and which affect its efficiency as an energy absorber, is the first step in any fundamental inquiry concerning the fertility of the sea.

The consideration of this cycle indicates that the fertility of any area

of the open oceans, not subject to considerable inflows of water from other areas, depends upon three main factors. (a) The length of time protein formed by phytoplankton remains part of the plant or nourished animals body. (b) The time which elapses during the decay and formation of ammonium salts and phosphate from corpses and excreta. To this must be added the time taken for nitrate forming bacteria to convert the ammonium into nitrates. (c) The time which elapses before the reformed nitrate and phosphate again reaches the upper layers where there is sufficient light for photosynthesis.

Dealing with these three factors in turn, the first is controlled by the natural length of life of the plants and animals, and by the proportion of the plants which are eaten. This decides the proportion of total living matter to the rate of loss by natural death. The proportion of total living matter to its rate of loss by respiration and excretion is controlled by *temperature*. In a warm sea the loss will be in excess of that taking place under colder conditions, since the rate of metabolism roughly doubles for a rise of 10°C ., and more food will be required by animals to provide for their irreducible metabolism necessary to maintain life.

Of the second factor (b) there is little information. Presumably the breakdown products of proteins and phosphoproteins produced in the course of metabolism are excreted mostly as urea and phosphates, and the decay of corpses is largely brought about by bacterial agency. From the analyses of water collected in the Atlantic during the expedition of the *Planet* it appears that there is rarely less than 25 milligrams per cubic metre of ammonium nitrogen in the waters of the open ocean (15, Tafel 9). The distribution of nitrate forming bacteria, possibly identical with the *nitrobacter* of the land (7) (13) in the open ocean is quite unknown.

The third factor (c), being the length of time that the reformed nitrate lies unusable below the illuminated upper layers, is of great magnitude. A glance at Table IV shows an enormous store of nitrate below the upper 100-metre layer. A rough calculation indicates an amount somewhere in the order of 250 thousand million metric tons of nitrate-nitrogen in the deeper layers of the great oceans, lying dormant until such time as currents bring the deep water into the upper sunlit layers. There is every reason to suppose that this third factor regulates the speed at which nitrogen and phosphorus pass through the complete cycle in the sea *as a whole*, being the slowest in the series of changes.

In particular shallow areas, however, such as where tidal streams and sufficient surface cooling to set up convection currents reaching to the bottom cause vertical circulation, during the whole or part of the year, the regenerated nitrate is subject to no delay before again becoming available. These areas, usually coastal, are very fertile. The North Sea,

English Channel, and the Shallow Shelf between the Grand Banks and Cape Cod are such. Here the conditions are somewhat complicated by a certain amount of inflow of oceanic water, and by the fact that the rate of regeneration of nitrate, and phosphate, from dead organisms overtakes the rate at which plant life utilises it during the autumn and short days of winter. A store of available nitrate and phosphate (16) is thus formed which is rapidly used up as soon as some three hours of sunshine per day occur in the spring in the English Channel. Insufficient illumination becomes the limiting factor during the winter months, delaying for a period the rate at which combined nitrogen and phosphorus passes through the complete cycle.

In the deep open oceans the winter cooling of the surface layers in temperate and arctic regions will set up convection currents. By lessening the density gradient, the convection currents will be assisted by wave motion in bringing about more effectual mixing with the layers below. Hence in such latitudes, as opposed to the subtropical regions, a small store of nutrient salts may be expected in the spring, and in general a richer plankton particularly in the early summer. In the tropical regions of the Atlantic the heated surface water streams away to form the Gulf Stream, etc., to be replaced by water upwelling from below. Hence in these regions richer plankton may be expected than in the subtropical. These expectations are born out by the results of Hensen's Plankton Expedition (14).

It is a remarkable fact that plant growth should be able to strip sea-water of both nitrates and phosphates, and that in the English Channel the store of these nutrient salts formed during autumn and winter should be used up at about the same time.

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SUMMARY.

1. A method of estimating nitrates in sea-water is described.
2. A considerable concentration of nitrates occurs in the depths of the ocean and a very low concentration (0 to 10 parts per 1000 million of nitrate-nitrogen) in the surface layers of those areas where there is no upwelling of water from below.
3. The nitrates in the water of the English Channel, twenty-two miles south-west of Plymouth, are almost entirely utilised by phytoplankton in the summer, and commence to be reformed from ammonium salts at a greater rate than they are utilised by the end of the summer. The

nitrate reformed near the bottom mix with the upper layers when the column of water becomes nearly isothermal in the autumn.

4. Sea-water from near the bottom at this position contains nitrate-forming bacteria.

5. Conditions which limit the total plant and animal population of areas of the sea are discussed.

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