The Organic Metabolism of Sea-water with Special Reference to the Ultimate Food Cycle in the Sea*

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With 5 Figures in the Text.

Introduction.

It has long been known that, of the total organic matter in the sea, the major portion is not incorporated in the cell substance of animals and plants nor even in the detritus of their decomposition, but exists in solution in the sea-water. Pütter (e.g. 1907, 1909) endeavoured to show that this dissolved organic matter plays a central rôle in the nutrition of the living organisms in the sea.

The arguments, experiments and observations *pro* and *con* regarding this important problem have been reviewed by Krogh (1931) and others (see, e.g. Bond, 1933). Krogh assembled from his own researches and those of other workers a chain of evidence which makes it appear that none of the macroscopic organisms, nor even the larger microscopic forms, can subsist to any appreciable extent on the dissolved organic matter. Bond's (1933) observations are in agreement.

There remains the question to what extent *bacteria* may utilise the dissolved organic matter in sea-water. This is of considerable importance because of the undoubted fact that bacteria may serve as food for larger organisms such as protozoa. It is known, also, that there are indeed few natural organic substances which are not attacked by some bacteria and that bacteria can extract nutriment from extremely dilute solutions of some organic compounds. How far these two statements can be combined in discussing sea-water is uncertain.

Our problem in reality is two: (1) How great are the metabolic activities of bacteria in sea-water under the various conditions of the laboratory? (2) What bacterial activities may be inferred to take place in the sea itself?

* Contribution No. 79 from the Woods Hole Oceanographic Institution.

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The chemical difficulties in the way of direct determination of the dissolved organic substances have been insuperable until recently, and still prevent a practicable direct measure of the degradation of the dissolved organic matter by bacteria. However, the methods of Krogh and Keys (1934) have shown that the organic matter in sea-water is generally of the order of 200 mg. nitrogen and 6 to 10 times that much carbon per cubic metre (Krogh, Keys and Christensen, 1935). The oxidation of any appreciable fraction of this by metabolic activities of organisms should be revealed by a change in the content of dissolved oxygen in the water.

The laboratory examination of the oxygen content of plankton-free sea-water under different conditions after collection is one tool we have used. A second consists in the study of the bacterial population of the water in experiments parallel to the oxygen studies. A third attack was in the direction of an effort to discover what substances, normally present in the sea, may be, by virtue of low concentration, limiting the bacterial use of the dissolved organic matter. We have studied ammonia, nitrate, nitrite, phosphate, and, to a limited degree, hydrogen and hydroxyl ions. In this category we may also place studies at different temperatures. Finally, the application of these results to the elucidation of the actual events in the sea requires consideration of the oxygen, bacterial populations, temperature, etc., in the sea-water *in situ*.

Methods.

To remove all plankton, including nannoplankton larger than bacteria, we have used filtration in a Büchner funnel through two thicknesses of the most retentive filter paper—Whatman's No. 44 or No. 50. To give comparisons with the filtrations of other workers, we have used a single thickness of Whatman's No. 1 or No. 2 paper. The removal of all particulate matter, including bacteria, was accomplished by filtration through a Zsigmondy type collodion membrane filter. A few small samples were prepared free of colloids by ultra-filtration through a membrane filter designed by one of us (Krogh).

Oxygen determinations were made by the Winkler method, with all titrations in duplicate. In the experimental series, the procedure was carefully to fill by siphon, with preliminary flushing, a series of identical glass-stoppered bottles. These were stored in the dark under water and bottles withdrawn, in duplicate, from time to time for analysis. The duplicate results agreed to within 0·02 c.c. O_2 per litre and better in almost all cases.

Bacterial populations were estimated from agar plate counts on at least 3 plates. Several dilutions were always made so that the cells per plate were always within the range 20 to 300. The medium used was

kindly supplied by Dr. S. A. Waksman. It had the following composition :

Glucose . . .	1 gm.
Peptone . . .	1 gm.
K_2HPO_4 . . .	0·5 gm.
Agar . . .	10 to 15 gm.
Sea-water . . .	1000 ml.

The plates were counted after incubation at 21 to 23° C. for 2 days and re-counted at the end of a week.

Ammonia determinations were made by the method of Krogh (1934). The reduced strychnine method of Harvey (1926, 1928) was used for nitrate, and nitrite was estimated by the Griess-Ilosvay method as used by Rakestraw (1933).*

Sub-surface samples were taken by cleaned Nansen bottles. It should be mentioned that throughout all the work meticulous pains were taken to prevent any kind of contamination.

In many cases the filtration was quickened by vacuum. The water was always subsequently thoroughly aerated by shaking over a period of 15 minutes or more. In low temperature experiments both water and bottles were pre-cooled before bottling and storage. This is necessary if the formation of air bubbles in the bottles due to cooling and the contraction of the water is to be avoided.

Oxygen Consumption of Stored Sea-water.

It has long been known that when sea-water is stored out of contact with the air in the dark there is a diminution in the oxygen content. In most cases, however, either the water was not carefully filtered (see, e.g., Knauthe, 1898)—it should be borne in mind that single filtration removes only a portion of the smaller plankton—or the exclusion from the air was brought about by a layer of oil (e.g. Winterstein, 1909), or other possible sources of error were not excluded.

We found, similarly to Gran and Ruud (1926) and Föyn and Gran (1928), that when fresh sea-water is brought into the laboratory and stored at 20 to 23° C., there is a rapid bacterial development and reduction of oxygen content. Figure 1 gives the results of two experiments with water from Woods Hole Harbour. The "respiration" in the doubly filtered water was considerably less than in the raw water, but in both cases the oxygen removal was rapid in the first 2 or 3 days and thereafter slower, being very slow indeed after 10 or 15 days.

True ocean water behaves similarly. Experiments with water collected

* We are indebted to Mr. Henry Mahnke for making the nitrate and nitrite analyses.

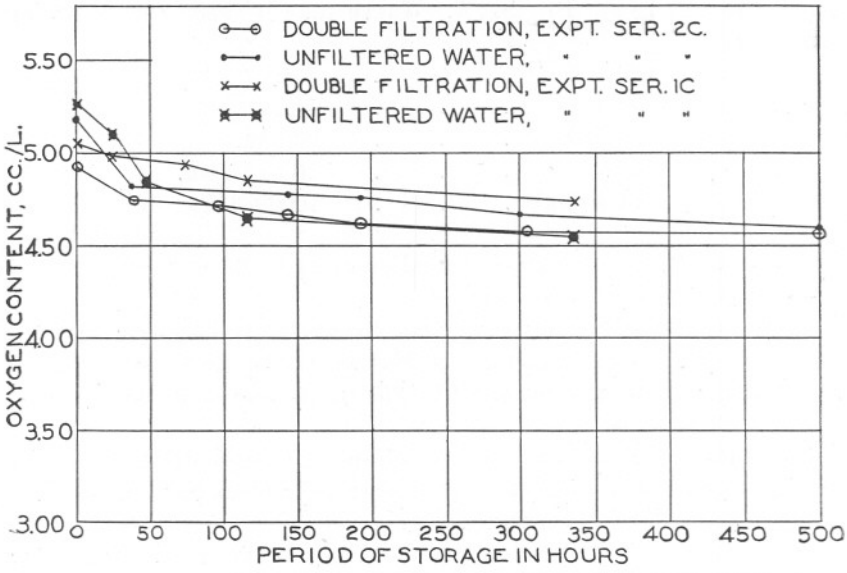

FIG. 1.—" Respiration " of sea-water stored in the dark at 20 to 23° C. Water from Woods Hole Harbour.

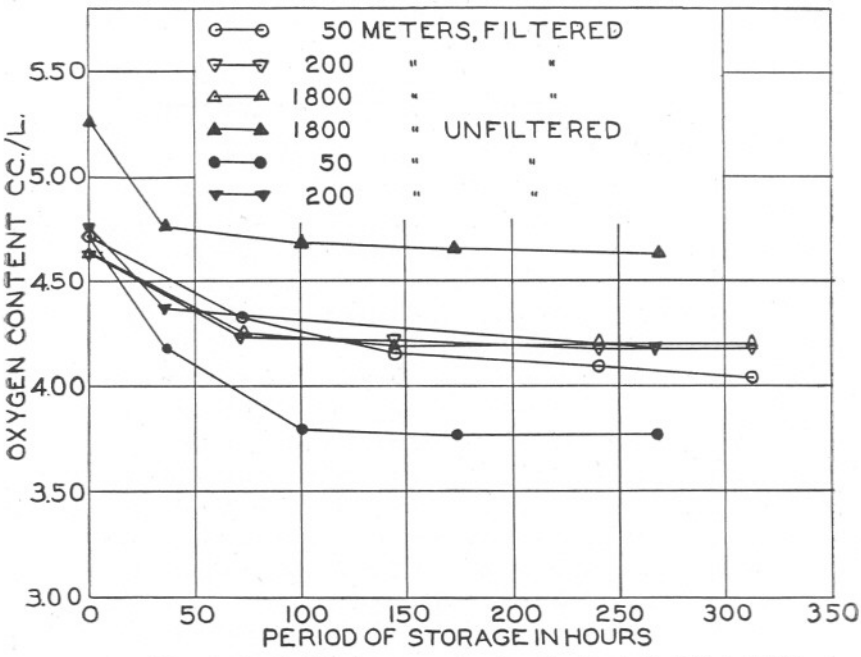

FIG. 2.—" Respiration " of true ocean water stored in the dark at 21 to 23° C.

at various depths at the 1000-fathom line south of Martha's Vineyard are summarised in Figure 2. Some differences in the " respiration " of waters from different depths appear; respiration of the water from within the photosynthetic zone is considerably more active than in the deeper water.

In Figure 3 is given the respiration observed in three series of experiments carried out under identical conditions with waters from Woods Hole, Martha's Vineyard and the open ocean respectively. The initial oxygen content in all these was between 4·8 and 5·2, and they have all been corrected to a standard initial value of 5·00 c.c. to facilitate comparison. Here, as in all our experiments, it is clear that a large fraction of

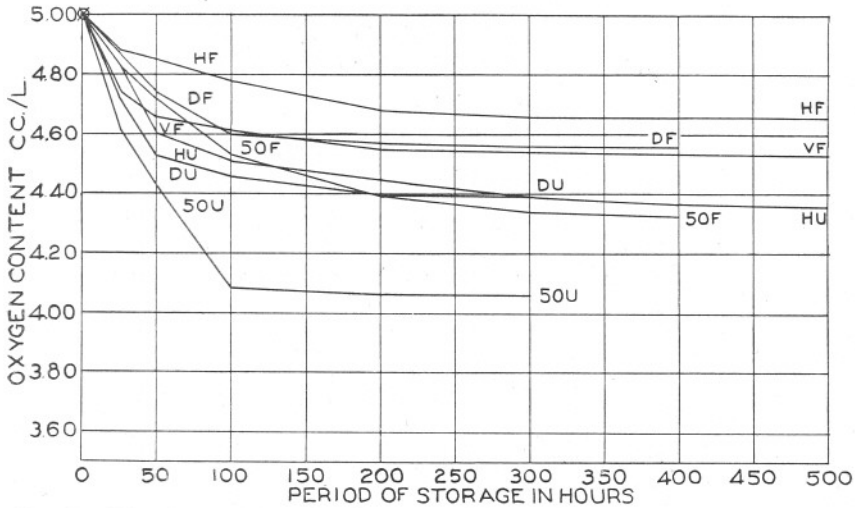

FIG. 3.—" Respiration " of various sea-waters stored in the dark at 21 to 23° C. HF= filtered Woods Hole Harbour water, UF=the same unfiltered, VF=filtered water from Vineyard Sound. 50F=sea-water from the open sea, 50 metres' depth, filtered; 50U=the same unfiltered. DF=water from the same station as 50 F but from a depth of 1800 metres, filtered; DU=the same unfiltered. Water from 250 metres at the same station behaved so nearly exactly alike DU and DF that the values are not given in this figure.

the total respiration of raw water must be ascribed to the decomposition of contained plankton. And here also both filtered and unfiltered waters from 50 metres' depth show the highest respiration.

Discussions by previous workers on this subject have been based on the belief that simple filtration produces a filtrate which is " plankton-free." Although Lohmann (1908, 1911) in particular recognised the fact that nannoplankton largely escape in ordinary filtration, the fact that the nannoplankton forms a large fraction of the total mass of plankton has not been sufficiently recognised. Allen (1919) concluded that Lohmann's estimates for the nannoplankton were far too low owing to the inefficiency of the centrifuge method of separation. Bond (1933) found that of the

total mass of plankton the nannoplankton may average a third and at certain seasons may represent an even greater fraction of the total. With this in mind we adopted the double filtration technique described in the " methods " section of this paper, and comparison experiments indicate to how great an extent ordinary filtration may err in producing " pure " sea-water.

In Table I are compiled the average respirations in filtered water prepared by filtration through a single thickness of ordinary filter paper and by our double filtration method.

TABLE I.

Oxygen Consumption in Oceanic Water Singly and Doubly Filtered.

Series A at 5° C. throughout, Series B at 21–22° C. Consumption in cubic millimetres O_2 per litre for the indicated period.

	Series A.					Series B.	
	0 to 100 hrs.	100 to 200 hrs.	200 to 300 hrs.	300 to 400 hrs.	400 to 500 hrs.	0 to 100 hrs.	100 to 200 hrs.
Single filtration	60	90	110	20	10	490	50
Double filtration	30	40	50	20	10	370	60

It is obvious that when filtration methods less efficient than ours are used a very large fraction of any total respiration observed subsequently must be ascribed to the decomposition of nannoplankton.

The Effect of Temperature on Sea-water " Respiration."

A striking difference in the respiration of sea-water stored at low and at ordinary temperature is apparent in Table I. This difference is consistently observed and is illustrated in Figure 4, which gives results from experiments with harbour water (Woods Hole) and inshore water (Vineyard Sound). Especially is the initial respiration very much greater in the warmer water. Whether eventually the total respiration in the cold and in the warm water would reach the same level is uncertain ; up to 3 or 4 weeks the difference is still maintained.

In water from the deep ocean we observed an interesting and perhaps significant phenomenon. When water was collected from several thousand metres' depth and never allowed to warm up, the oxygen consumption was much less than in some of the same water which warmed to 22° C. for only a few hours and then was cooled to the same level as the other

water. In 2 experiments of this sort we found that in 450 hours' storage at 5 to 6° C., water which had never been allowed to rise above 7° before the experiment showed less than half the oxygen consumption of another portion of this water which had risen to room temperature for a few hours on board ship. Water collected from 2000 metres, doubly filtered and stored at 5 to 6° C., which had never been allowed to warm during any of the operations, showed a total respiration of less than 0·02 c.c. per litre in 150 hours.

It appears, then, that even temporary warming has a marked effect in producing conditions favourable to bacterial development and chemical

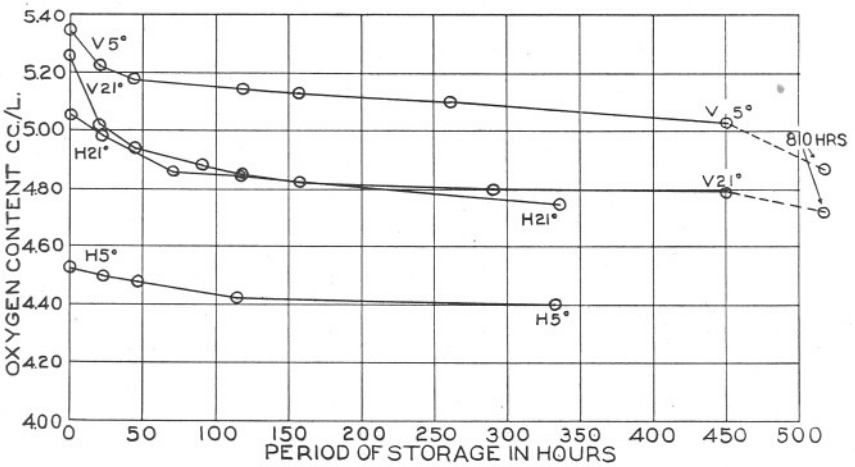

FIG. 4.—" Respiration " of sea-water from Woods Hole Harbour and from Vineyard Sound stored at 5° and at 21°. V refers to Vineyard Sound water, H to Woods Hole Harbour water.

decomposition in sea-water. This is one factor which is important in the attempt to study the organic matter of the sea by laboratory experiments.

THE BACTERIAL POPULATION.

Early studies on the bacterial population of various sea-waters *in situ* showed that the numbers decrease very abruptly with the distance from shore (Russell, 1892, 1893), and this finding has been confirmed by many workers (see Benecke, 1933). Reuszer (1933) found numbers ranging from a few hundreds per c.c. down to almost zero in waters a few miles south of Cape Cod. In the open sea, 50 miles or more from land, it appears that at all depths fewer than 100 cells per c.c. will develop on plating. Direct microscopic examination of sea-water reveals two or three hundred times as many bacteria (Cholodny, 1929; Rasumov, 1932; Waksman and Carey, 1935); it seems, however, that the number of cells viable on agar plates is roughly proportional to the total.

A remarkably rapid increase in bacterial numbers follows withdrawal of a sample of sea-water from the sea. Typical results obtained from inshore water are shown in Figure 5.

Results qualitatively similar to those given in Figure 5 were obtained in a large number of experiments. In each case a maximum bacterial

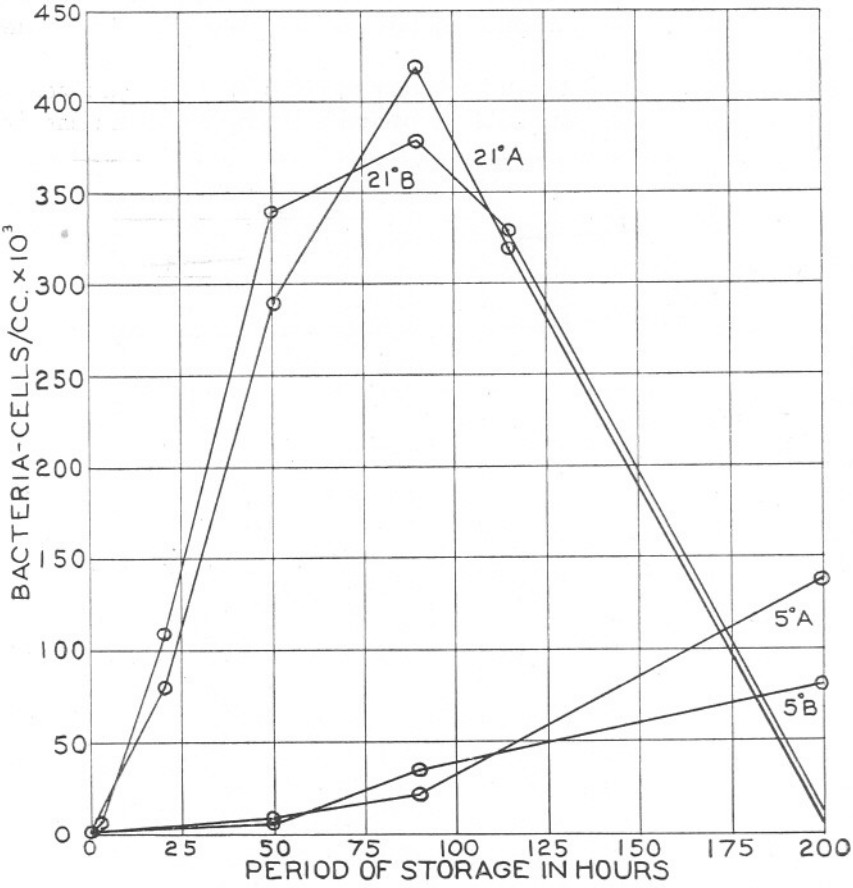

FIG. 5.—Development of bacteria in sea-water stored in the dark at 5° and at 21° C. Plate cell counts.

population was found in 3 or 4 days at 21°, and at 5° the maximum was not reached for several weeks. However, the bacterial counts from experiment to experiment showed greater variation than did the oxygen consumption in the experiments discussed in the preceding sections. This prompts the question—to what extent is the bacterial population, either the total numbers or the rate of increase, reflected in the oxygen consumption ?

In our experiments, at least, there was only the crudest indication of a relation between bacteria and oxygen consumption. When the bacterial counts are high, the oxygen generally disappears more rapidly than when only few bacteria develop on the agar plates. Table II illustrates results from two parallel experiments with harbour water and may be taken as typical.

TABLE II.

Relation between Bacterial Development and Oxygen Consumption in stored Sea-water.

A refers to the first experimental series, B to the second.

Time Period.	Sample.	Temp.	O_2 used in c.c.	Average cells/c.c.	Increase cells/c.c.	O_2 uptake per 100,000 cells, per 100 hrs.	O_2 uptake per 100 hrs. per increase of 100,000 cells.
0 to 50 hours	A	5°	0·18	2,600	3,210	13·8	11·6
	A	21°	0·32	170,000	337,000	0·38	0·18
	B	5°	0·17	1,400	1,960	24·2	17·4
	B	21°	0·34	147,000	294,000	0·50	0·22
50 to 115 hours	A	5°	0·03	20,000	18,000	0·23	0·26
	A	21°	0·11	360,000	130,000	0·05	0·12
	B	5°	0·04	37,000	34,000	0·17	0·18
	B	21°	0·05	360,000	40,000	0·02	0·18

In sterile experiments, the oxygen content of the stored water was not always constant, but the maximum " consumption " was never more than 0·05 c.c. no matter how long the samples were stored.

It was a constant peculiarity that in the first few days in low temperature experiments, the respiration, though small in total, was always very large per unit of viable cells. Unless the plating methods give a grossly erroneous picture, the results seem to indicate that bacterial respiration proper can be responsible only for a fraction of the oxygen used up, while the rest which disappears fairly rapidly, even at low temperature, must be accounted for in some other way.

In two experiments small amounts of sea-water were prepared, by ultrafiltration, free of all particulate matter, including colloids. These samples were inoculated with non-sterile sea-water and bacterial counts made from time to time. The multiplication of bacteria in this water was at least as great as in ordinary filtered water. The samples were too small to permit studies of the oxygen contents.

Influence of Added Ammonia and Phosphate.

In a large number of the experiments ammonium sulphate was added to portions of the water to give a final concentration of 50 to 75 gammas

ammonia nitrogen per litre (i.e. 3 or 4 times the control). In almost all cases there was no effect on either oxygen consumption or bacterial growth.

In a few cases there was an indication of a depressant effect, but this was so slight as to be doubtful. Clearly the changes in the sea-water are not, in these experiments, limited by lack of available nitrogen.

The addition of dipotassium phosphate in amount equivalent to 100 gammas phosphate per litre had little or no effect in experiments with deep ocean water (which has naturally a high phosphate content). With harbour water, however, there was certainly an increased respiration, most marked in raw water, but also noticeable in filtered water in the first few days. Table III shows the total respiration in an experiment of this sort. Almost identical results were obtained in a duplication of the experiment.

TABLE III.

INFLUENCE OF ADDITION OF 0·1 MG. PO_4 (AS K_2HPO_4) TO SEA-WATER TAKEN FROM WOODS HOLE HARBOUR.

Water stored in dark at 22° C.

Material.	Raw water.	Raw water + PO_4.	Filt. water.	Filt. water + PO_4.
	Total Respiration first 100 hours.			
O_2 used, c.c./l.	0·34	0·69	0·23	0·32
	Total Respiration first 500 hours.			
O_2 used, c.c./l.	0·70	1·30	0·38	0·52

ALTERATIONS IN AMMONIA IN STORED SEA-WATER.

The oxygen consumption and bacterial development observed in the present experiments indicate metabolic processes which would be expected to involve also nitrogen compounds. Two diametrically opposed expectations could be developed theoretically. It would seem reasonable that in the bacterial development ammonia, as an easily available form of nitrogen, would be used and therefore the ammonia content of the water would diminish. Equally reasonable would be the expectation that the bacterial activity in decomposing complex organic substances in solution in sea-water would liberate a certain fraction of the nitrogen as ammonia, and hence the stored water would show an increase in ammonia concentration. In actuality, neither of these possibilities is realised generally to a preponderant degree, and it would seem that both types of nitrogen metabolism take place in sea-water.

The more frequent behaviour of the ammonia concentration is to diminish on storage, especially when the initial value is high. In Table IV are assembled results showing this phenomenon.

TABLE IV.

AMMONIA CONCENTRATION OF STORED SEA-WATER, IN MG. NH_3–NITROGEN PER CUBIC METRE.

Experiments on board the *Atlantis* in the Gulf of Maine, September, 1933. Storage at 5° C. in dark. Sterile series by mercuric chloride (1/10,000); sterility checked by plating.

Sample.				
A	Time, hrs.	2·5	8·5	290
	NH_3 γlitre	23	19	8
B	Time, hrs.	6	18	90
	NH_3	49	28	23
C	Time, hrs.	1	7	290
	NH_3	39	41	10
A	Time, hrs.	2	9	290
sterile	NH_3	22	16	8
B	Time, hrs.	6	18	90
sterile	NH_3	44	32	27

The decreases in ammonia in the sterile series show that a purely chemical reaction is involved. Similar reduction in ammonia in sterile sea-water was observed by Kreps (1934), who also found, as we did, that the ammonia concentration in stored sea-water behaves in anything but a consistent fashion from experiment to experiment. Table V following contains results which illustrate other types of behaviour.

TABLE V.

AMMONIA CONCENTRATION OF STORED SEA-WATER, IN MG. NH_3–NITROGEN PER CUBIC METRE.

Water from Vineyard Sound, August, 1934. Storage in dark under water. In the A series the water was merely filtered free of plankton, in the B series ammonium sulphate was added initially. The temperatures are those at which storage took place. All values are averages of several analyses.

Time (hrs.).	1	20	54	111	204
A, 5°	18	—	30	21	17
B, 5°	54	—	40	32	27
A, 21°	18	11	6	21	27
B, 21°	54	44	25	39	35

The presentation of further data on ammonia concentrations in stored sea-water would only emphasise the fact that the phenomena are complex and are not as yet capable of precise interpretation. It should be pointed out, however, that in sea-water sterilised by filtration and stored in the dark at 20–22° C., ammonia liberation occurred in both our series of experiments of this kind, one of which is given in Table VI.

TABLE VI.

AMMONIA IN FILTRATION-STERILISED SEA-WATER FROM WOODS HOLE HARBOUR STORED IN DARK AT 20–22° C.

Time in hours.	3	20	72	115
NH_3, mg. N/cu. metre	10, 10	16, 17	21, 26	24, 26

Attempts to follow other forms of nitrogen reveal no changes of significant magnitude to throw light on the nitrogen transformations in stored sea-water. The experimental data summarised in Table VII are typical.

TABLE VII.

NITROGEN COMPOUNDS IN SEA-WATER, ALL VALUES IN TERMS OF MG. N PER CUBIC METRE.

Water from Vineyard Sound, filtered and stored at 8° C. in the dark. Series B with added ammonium sulphate.

	NO_2.	NO_3.	NH_3.	O_2 (c.c./l.).
A, start	1·3	55	18	5·23
A, 100 hrs.	1·1	52	20	5·14
B, start	2·2	55	45	5·24
B, 100 hrs.	2·2	54	31	5·12

It appears to us that the only conclusion that can be drawn with regard to nitrogen metabolism in stored sea-water is that there are usually a number of forces at work, some operating to reduce the free ammonia, others tending to release ammonia ; the observed ammonia concentration will always be a resultant of these forces, and the predominating action will be dependent, among other things, on the temperature, the varieties of bacteria present, and the chemical nature of the organic substances in solution, if this is variable.

DISCUSSION.

The bacterial development and the oxygen consumption observed in the present experiments can only have proceeded at the expense of organic nutriment gained from the dissolved matter in the sea-water. The amount of dissolved organic matter used in this way cannot be estimated except by making assumptions with regard to the RQ (ratio of carbon dioxide produced to oxygen consumed) characterising the bacterial metabolism, but it is not insignificant.

From analogy with aerobic bacteria in general, an RQ between 0·5 and 1·0 would be reasonable to ascribe to the mixed marine bacteria with which we are concerned. While it must be emphasised that we have no direct estimate for the true RQ, it may be illuminating to apply the best figure we can suggest on the basis of knowledge of the RQ of other forms and thereby calculate a *possible* figure for the amount of dissolved organic matter degraded in one of our typical experiments. In the experiment A 21° (see Table II) the total oxygen consumption per litre of sea-water in 115 hours was 0·43 c.c. at N.T.P. (760 mm. Hg and 0° C.). Assuming an RQ of 0·85 this would mean that $0{\cdot}85 \times 0{\cdot}43 = 0{\cdot}36$ c.c. CO_2 was produced. But 1 litre of CO_2 at N.T.P. weighs 1·96 grams so there has been produced 0·71 mg. CO_2. This means that in each litre $\frac{12}{12+2(16)} \times 0{\cdot}71$ or 0·19 mg. carbon has been degraded from dissolved organic complexes by the bacterial metabolism. Since the total dissolved organic carbon is of the order of 2 mg. per litre, roughly 10 per cent has been decomposed.

Similar calculations for our various experiments indicate that up to something like 10 or 15 per cent of the total dissolved organic matter was destroyed.

This much of the total dissolved organic matter, then, is readily susceptible to biological degradation, provided the stimulus of handling the sea-water in the laboratory is supplied. That such a "stimulus" is necessary is shown by the fact that, in the sea, neither the bacterial multiplication nor oxygen consumption can be observed to take place. It may be possible, of course, that in the sea protozoan populations keep pace and by feeding on the bacteria restrict them to small numbers. More likely would it be that there is always only a very small bacterial activity in pure sea-water owing to the extreme stability of the ocean as a chemical and physical environment.

It is certain that sea-waters of great variety—we studied harbour, inshore, offshore, and open ocean sea-water from depths of 1 to 2000 metres—all possess a great reserve of potential nutriment which suddenly comes into service when what seem to be very small alterations in the system are introduced. It is uncertain where this energising action takes place,

in the chemical nature of the organic complexes or in the bacteria themselves, but without it the metabolic activities would at least proceed at a far slower rate.

The fact that the burst of metabolic activity observed in the sea-water is always self-limiting may be interpreted in either of two ways. One possibility is that the bacterial development stops because the bacteria produce metabolites which are eventually toxic to them. The other possibility is that the bacterial metabolism ceases because only a small fraction of the total dissolved organic matter is available. In support of this latter suggestion is the fact that addition of small amounts of a great variety of organic substances results in a much greater bacterial activity. This is evident from our experiments with unfiltered water and has been demonstrated in experiments at Woods Hole by Dr. S. A. Waksman who studied the effect of addition to sea-water of a great variety of organic compounds.

The bacterial activity is ordinarily not limited by nitrogen want, but if pure carbohydrates are added to sea-water the bacterial metabolism ceases at a point where, although two or three times as much oxygen is consumed as without the carbohydrate, there is still a plentiful reserve of both carbohydrate and oxygen. The further addition of small amounts of nitrogen in any form with the amino linkage is followed by continued bacterial activity until all the oxygen is used up from the sea-water.

All this makes it seem likely that the principal limitation to bacterial development in pure sea-water is lack of *available* organic carbon, and that some of the dissolved organic matter can satisfy this want if the physical characteristics of the environment are slightly altered for a time. It is not improbable that this happens in the sea at various times and places, but that, in general, an equilibrium is maintained in which only a minimal bacterial population is supported by the dissolved material which comes to be, perhaps not wholly out of organic circulation, but certainly not ordinarily a major link in the ultimate food cycle in the sea.

SUMMARY.

Studies are reported of the behaviour of stored sea-water with regard to oxygen, ammonia, and bacteria content in relation to the conditions of storage and to the effect of various filtration procedures.

When sea-water is sterilised by filtration and stored in the dark, the oxygen content remains constant or diminishes only by less than 0·07c.c. per litre in several hundred hours.

In non-sterile experiments there is always an oxygen consumption roughly parallel to a bacterial multiplication which begins very suddenly

after collection of the water. These effects are greatest in " raw " water, less in paper-filtered water and least in water which is doubly filtered.

These metabolic activities are self-limiting and stop when there is still a large reserve of ammonia and oxygen and when only a small fraction of the dissolved organic matter has been decomposed.

The behaviour of the ammonia content in these experiments indicates that the ammonia content observed in sea-water is a resultant of purely chemical actions tending to diminish the ammonia and of biological forces in which both ammonia production and ammonia liberation by bacteria may be observed.

Great bacterial development may take place in sea-water freed of *all* particulate matter including colloids.

It appears that in the sea there is generally an equilibrium such that only minimal bacterial activity at the expense of dissolved organic matter may take place, but that very small changes in the system may make available for bacterial metabolism perhaps 10 or 15 per cent of the total dissolved organic matter.

Bacterial activity in sea-water is very sensitive to temperature and to alkalinity and relatively independent of ammonia, nitrate or nitrite and also oxygen above half-saturation. Lack of phosphate may on occasion be a limiting factor.

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