A Contribution to the Quantitative Study of Plankton.

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THE well-known work of Hensen (1887) was the first serious attempt to determine the actual number of individual plankton organisms in sea-water. Hensen's method consisted in straining a vertical column of water through a net of fine-meshed bolting silk having 6,000 to 6,500 meshes per square centimetre, the average length of the side of a mesh being 50 μ. A carefully measured sample of the organisms retained by the net was taken and the number of organisms in it counted. From this, since the area of the mouth of the net and the distance through which it was drawn were known, the number of organisms in a unit volume (1 litre) of water could be calculated, with the help of a coefficient of filtration for the particular pattern of net employed, which was determined experimentally. Many small organisms, however, escaped through the meshes of the net, and Lohmann (1902, 1908) made a special study of these by filtering through hard filter paper or closely woven taffeta silk, by an examination of the filtering apparatus in the "houses" of Appendicularians, and finally, for the quantitative estimation of the smallest organisms of all, by subjecting samples of sea-water to the action of a centrifuge making 1,400 revolutions per minute for a period of 7 minutes. The use of the centrifuge has been continued by Gran (1915) and by Lebour (1917). For the full literature of the subject the reader is referred to Gran (1915) and Lohmann (1911).

In the course of my work on the cultivation of plankton diatoms (1910, 1911) I became convinced that even the quantitative method based on the use of the centrifuge fell very far short of giving the total number of organisms actually present in a sample of sea-water, and the results recorded in the present paper show that this is certainly the case. The figures now given, which are based on culture experiments, though very greatly exceeding those obtained by the use of the centrifuge, must still be regarded as minimal figures, and by no means represent the actual number of organisms present, even when we leave out of consideration the bacteria, which I have not attempted to enumerate.

The culture solution used was the modification of Miquel's solution

recorded in the paper by Allen and Nelson (1910). Two solutions were made up :—

A.	Potassium nitrate	22·2 grams.
	Distilled water	100 grams.
B.	Sodium phosphate ($Na_2HPO_4 12H_2O$)	4 grams.
	Calcium chloride ($CaCl_2 6H_2O$)	4 grams.
	Ferric chloride (melted)	2 cc.
	Hydrochloric acid (pure concentrated)	2 cc.
	Distilled water	80 cc.

To 1 litre of sea-water 2 cc. of solution A and 1 cc. of solution B were added. After the addition of the two solutions, the sea-water was brought to the boil and then allowed to cool and stand for at least 24 hours, generally for some days, the precipitate which forms settling to the bottom. The clear liquid was poured off and used in the experiments. Long experience has shown that no growth of organisms (other than bacteria and possibly moulds introduced from the air) occurs in sea-water so prepared unless it is inoculated with sea-water containing such organisms. All flasks were thoroughly cleaned and baked in an oven before use, and pipettes were always both boiled and baked, a clean and sterile pipette being used each time a flask was examined.

On September 6th, 1918, a sample of sea-water was taken near the Knap Buoy, which is situated about ½ mile outside the Plymouth Breakwater. The sample was taken in a sterilised Winchester quart bottle, which was plunged, after the stopper had been taken out, one or two feet below the surface and allowed to fill. The sample was dealt with immediately it reached the Laboratory ; (1) by centrifuging 40 cc. of it twice, and counting the number of organisms under the microscope in the ordinary way and (2) by a culture method in which the organisms present in ½ cc. were allowed to grow.

1. EXAMINATION BY MEANS OF THE CENTRIFUGE.

This part of the experiment was carried out by Dr. Lebour, on the same plan as that usually followed by her in the quantitative determination of the microplankton of Plymouth Sound (Journal M.B.A., Vol. XI, p. 133, 1918).

Four glass tubes with pointed ends, each containing 10 cc. of the sea-water, were subjected for 10 minutes to the action of a centrifuge worked by a small water-motor and running at about 1,140 revolutions per minute.[1] The bulk of the water was then poured off from the tubes and

[1] The centrifuge was the same and worked at the same speed as that used by Dr. Lebour in her work on the microplankton of Plymouth Sound. The tubes were 65 mm. long (including the pointed ends) with an internal diameter of 18 mm. At a later date a careful comparison was made between the results given by this centrifuge and those given by a hand centrifuge driven at 1,680 revolutions per minute, with two tubes 95 mm. long (including the pointed ends) and 11 mm. diameter. The former, driven by the water-motor, proved to be distinctly more efficient.

the drop containing the organisms remaining in the pointed end of each tube was removed by means of a pipette and transferred to a ruled glass slide on which the number of organisms was counted under a microscope. The bulk of the water was then put back in the tubes, again centrifuged for 10 minutes and the organisms deposited, examined and counted.

Table I gives the result obtained by Dr. Lebour by using the centrifuge in this way.

Table I. Water Sample from near Knap Buoy, September 6th, 1918. Four tubes of 10 cc. each, centrifuged twice.

AVERAGE NUMBER IN 10 CC.

× indicates that the organism was seen once only in the 40 cc.

Navicula sp.	10	Lithodesmium undulatum	1
,, membranacea	1	Pleurosigma sp.	2
Thalassiosira gravida	2	Hyalodiscus stellatus	×
,, sp.	×	Prorocentrum micans	6
Chætoceras sp.	16	Peridinium sp. juv.	7
Skeletonema costatum	26	Glenodinium bipes	3
Nitzschia closterium	8	Amphidinium crassum	×
,, delicatissima	7	Gymnodinium teredo	×
,, seriata	1	,, sp. juv.	3
Coscinodiscus radiatus	1	Spirodinium glaucum	×
,, excentricus	×	Cochlodinium sp.	×
,, sub-bulliens	×	Flagellata indet.	21
Rhizosolenia faercensis	2	Tintinnopsis beroidea	1
Asterionella japonica	13	Infusoria indet.	×
Eucampia zoodiacus	3	Foraminifera indet.	×
Paralia sulcata	8	Algal spore	×
		Larval bivalve	×
		Larval crustacean	×

This gives **14·45 organisms per cubic centimeter or 14,450 per litre.**

2. EXAMINATION BY MEANS OF CULTURES.

The bottle containing the sea-water sample of September 6th, 1918, was shaken up and ½ cc. was drawn out and measured off with a 2 cc. pipette graduated in 50ths of a cc. The ½ cc. was added to 1,500 cc. of sea-water which had been treated with Miquel solutions as already described, boiled and allowed to cool and the precipitate to settle. After being well shaken up the solution was divided up into 70 very small flasks (capacity of flask 50 cc.), so that on the average there was a little over 20 cc. in each flask. These 70 flasks were placed in a north light and kept at room temperature without a fire (September and early October).

After ten days many of the flasks showed distinct signs of growth, and the first examination was commenced on September 16th and extended to September 19th. The different kinds of organisms found growing in each flask were recorded. A second examination was made between October 4th and October 15th, and a third examination between October 17th and October 23rd.

The organisms found were chiefly diatoms, flagellates and other protozoa. Bacteria were practically always present, but no attempt was made to distinguish them, and they are not included at all in the figures given below.

It must be understood clearly that the numbers given are *minimal* numbers. Closely allied species, where there was any possibility of their being merely varieties or " growth forms " of one species, were counted only as one organism when they occurred in the same flask. Thus *Nitzschia delicatissima* and *Nitzschia seriata* are not counted as distinct, and never more than one species of minute *Thalassiosira* is recorded from the same flask. In the case of *Chætoceras* never more than two were recorded in any one flask, and then only when the distinction between them was very marked. Flagellates were only regarded as distinct when their characters were very marked indeed.

The largest number of different kinds of organisms found in one flask was 7 ; in two flasks only one organism was distinguished, and there were no flasks in which no growth at all took place. The average number of different kinds of organisms per flask for the whole series was 3·3. Adding together the numbers found in each flask for all the 70 flasks we get a total of 232 different organisms. Now each of these must have been represented by at least one individual or unit, either as cell or spore, in the original $\frac{1}{2}$ cc. of sea-water from which the experiment was started. The sample of sea-water therefore must have contained **at least 464 organisms per cubic centimeter or 464,000 per litre.**

The real number must be very considerably greater than this. In the case of several of the minute diatoms, especially small *Thalassiosira*-like species, they form little patches of encrusted growth on the sides and bottom of the flask, and when the flasks are first examined, and before they have been disturbed in any way, two or three or more patches are frequently seen in the same flask, showing that the growth in that flask has probably started from more than one individual cell or spore. In the case of chain-forming diatoms such as *Chætoceras* it is practically certain that the chains are not all broken up into individual cells by the shaking given to the $1\frac{1}{2}$ litres of inoculated culture solution before it is distributed amongst the 70 small flasks. In many cases the growth of such species in a flask will have started from more than a single cell.

The organisms recorded are only those that will grow in the particular

culture medium employed, and under the conditions of light, temperature, etc., in which the experiment was carried out. It is certain that many species cannot be cultivated at all under these conditions. I have, for example, in the whole course of my work only once had a species of Peridinian in culture.

Taking all these points into consideration we should probably be within the mark in putting the number of organisms in the sample of sea-water examined at least one million per litre.

To what extent the number found represents individual cells and to what extent it represents "spores" it is impossible to say definitely. The organisms most frequently occurring in the cultures are small diatoms and flagellates of different kinds, the usual mode of reproduction in both cases being by binary fission, reproduction by spore formation being exceptional.

The following table gives the details of the experiment just described :—

Table II showing the minimum number of organisms (excluding bacteria) in ½ cc. of sea-water collected near the Knap Buoy, September 6th, 1918, as determined by a culture experiment in which it was distributed amongst 70 small flasks.

	Number of Occurrences in ½ cc.
Navicula sp.	4
Thalassiosira (large sp.)	9
,, (minute sp.)	59
Chætoceras sp. (not more than 2 recorded in one flask)	73
Skeletonema costatum	6
Nitzschia delicatissima	8
,, closterium	19
Coscinodiscus sp.	4
Detonula sp.	1
Rhizosolenia færœensis	2
Asterionella japonica	1
Eucampia zoodiacus	1
Minute diatom indet.	1
Chrysomonads	14
Cryptomonad	1
Peridinian	1
Coccolithophora	4
Tintinnids	2
Other Protozoa	22
Total	232

Notes on Table II.

Chætoceras sp. The most common species was a small one with cylindrical cells 11 μ long by 2·8 μ diameter. There was also a species with square cells, the side of the square being considerably less than the length of the cylindrical cells, probably about 5 μ, though no actual measurement was taken.

Thalassiosira sp. There were at least two minute species very frequent, one with diameter 4 μ and height 4 μ, the other with diameter 2·8 μ. These two were counted as one when occurring in the same flask. A larger species of *Thalassiosira* with diameter 8·3 μ was regarded as different.

Navicula sp. Two species occurred, a larger and a smaller one. The former had frustules 25 μ long by 5 μ broad at the widest part, and was the one usually found. The smaller one had frustules about 13 μ long.

The *Chrysomonads* were chiefly 3 μ to 7 μ in diameter, the *Cryptomonad* was 7 μ.

For comparison with the above experiment, a culture experiment started August 6th, 1918, and carried out in a similar way in 66 flasks, with $\frac{1}{2}$ cc. of sea-water from a sample taken near the Knap Buoy, may be recorded. In this case the experiment was commenced for another purpose and no comparative examination of centrifuged samples was made.

The number of different organisms proved in the 66 flasks, that is the minimum number of individuals which must have been present in the original $\frac{1}{2}$ cc. of sea-water, was 231, which is almost the same figure as that found from the experiment of September 6th.

The following table gives a list of the organisms. It will be noticed that the proportion of flagellates is larger in this sample than in the previous one.

Table III showing the minimum number of organisms (excluding bacteria) in $\frac{1}{2}$ cc. of sea-water collected near the Knap Buoy August 6th, 1918, *as determined by a culture experiment in which it was distributed amongst* 66 *small flasks.*

	Number of Occurrences.
Navicula (2 species) . . .	60
Thalassiosira sp.	36
Chætoceras sp.	18
Skeletonema costatum . . .	6
Nitzschia delicatissima . . .	17
,, closterium . . .	7

	Number of Occurrences.
Lauderia borealis . . .	1
Flagellates (not more than 2 recorded in one flask)	73
Ciliates	10
Amœba	1
Coccolithophora	2
Total . . .	231

A comparison of the list of species and number of specimens recorded from the centrifuged sample (Table I) with that obtained from the culture (Table II) shows a considerably larger number of *species* revealed by the centrifuge, as we should expect from the larger sample examined (40 cc. as against $\frac{1}{2}$ cc.), whereas the number of *individuals* per cc. revealed by the culture is very much larger, more especially in the case of the smaller species.

In Table IV, which is compiled from Tables I, II, and III, the numbers given by the three experiments have been multiplied up to show the number of organisms per litre, so that the three can be more readily compared.

Table IV. Number of plankton organisms per litre shown by the three experiments.

	Centrifuge. Sample of Sept. 6th. Number per litre from Table I.	Culture. Sample of Sept. 6th. Number per litre from Table II.	Culture. Sample of Aug. 6th. Number per litre from Table III.
Navicula . . .	1,100	8,000	120,000
Thalassiosira . .	225	136,000	72,000
Chætoceras . .	1,600	146,000	36,000
Skeletonema costatum .	2,600	12,000	12,000
Nitzschia delicatissima and seriata .	800	16,000	34,000
Nitzschia closterium .	800	38,000	14,000
Coscinodiscus . .	150	8,000	—
Rhizosolenia færœensis .	200	4,000	—
Asterionella japonica .	1,300	2,000	—
Eucampia zoodiacus .	300	2,000	—
Other diatoms . .	1,125	4,000	2,000
Peridinidæ . .	2,000	2,000	—
Other Protozoa . .	2,250	86,000	172,000
Totals .	14,450	464,000	462,000

There is obviously at present no method that is capable of giving us a complete quantitative estimate of the total number of individual organisms in a sample of sea-water, but by combining a number of method we shall gradually get nearer to the solution of the problem. The present note it is hoped may carry the question a step forward.

My best thanks are due to Dr. M. V. Lebour, not only for carrying out the examination of the sample of sea-water by means of the centrifuge, but also for constant help in the determination of species.

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