A SIMPLIFIED PLANKTON COUNTING METHOD

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The microscopic assemblage of life in lakes, ponds, streams and rivers, which is made up of both plant and animal forms, is known collectively as plankton. In 1882 Hensen was one of the first to attempt the study of these organisms quantitatively. His investigations were conducted on the Baltic and North seas with nets of various mesh sizes. It was soon found that even the finest meshed nets allowed many organisms to pass directly through.

Sedgwick (1888) and Rafter (1888), recognizing the inadequacy of the plankton net, both described methods of examining water microscopically after concentrating by filtration through sand supported on a bolting silk disk. This method has become known as the Sedgwick-Rafter method and is still widely used today.

In 1911 the extensive limnological program of Birge and Juday was started on the Wisconsin lakes and demanded a still more efficient and practical field plankton sampler. There evolved a portable centrifuge which is now known as the Foerst electric centrifuge. This instrument has been in use for more than thirty years and is recognized by the American Public Health Association, along with the Sedgwick-Rafter method, as the standard procedures for the examination of water and sewage (1946).

Several other devices or modifications, such as the Kofoid filter, the Juday plankton trap, the Clarke and Bumpus quantitative net sampler, have been developed and are in use. However, all require a considerable financial investment in equipment and supplies in addition to the microscope, which is the most fundamental. As a result, many may not have attempted quantitative work on the plankton because of the lack of what appears to be essential equipment. The writer believes that anyone possessing a microscope and an interest in the plankton is in a position to carry on a quantitative study of these organisms. This paper has been prepared with the beginner in mind and is not necessarily directed to the specialist in the field of biological productivity.

That it might be possible to secure reliable quantitative data quickly and without employing expensive equipment was first suggested by Baylis (1922) in a study of the microorganisms in the Baltimore water supply. In 1929, Baylis and Gerstein presented comparative counts made by the Sedgwick-Rafter and the Baylis direct count on the plankton in the Chicago water supply. The data indicated to the writer that the method deserved further consideration. Thus, while an employee of the City of Chicago working with both Baylis and Gerstein, routine plankton determinations were made with the Sedgwick-Rafter method, the Foerst centrifuge, and the direct count on an experimental basis.

TABLE 1

Order of Counts	Calculated No. of Organisms per ml from 10 areas	Percent Deviation from mean	Calculated No. of Organisms per ml from 50 areas	Percent Deviation from mean
1	1633	7	1962	8
2	2566	46	1555	15
3	1541	12	1700	7
4	1908	9	1695	7
5	2141	22	1805	1
6	1458	17	1927	6
7	1958	12	1860	2
8	991	44	1992	9
9	1933	10	1827	0.3
10	1416	19	1899	4
Mean	1755	20	1822	6

DIRECT COUNT METHOD

As the name implies, the direct count method does not require any concentration or special treatment of the water sample. A Sedgwick-Rafter counting cell or an improvised cell cut from an old piece of a tire inner tube and cemented to a glass slide serves the purpose very well. The usual inside dimensions of the Sedgwick-Rafter cell are 50 by 20 mm but for the direct count it is desirable to increase the area to at least 50 by 40 mm, which means doubling the volume ordinarily observed. Baylis recommends a 5 ml or even a 10 ml cell for making a rapid survey count in which the crustacean or larger organism population is established.

It should be noted that not even the Whipple ocular micrometer is required for the direct count, for instead of counting individual fields the whole length of the cell is traversed and the organisms encountered are enumerated. A minimum of 100 sq mm or two traverses of the 50 mm counting cell are recommended. Four strip counts across a 2 ml cell will increase the accuracy of the method. It is even more desirable to count 100 sq mm in two different 1 ml portions of the original common sample, and such practice should be encouraged. The time required to perform these strip counts is still less than it takes to prepare the samples and determine the count by either the sand filtration or the centrifuge methods.

The factor used for converting a concentrated sample count into actual numbers of organisms per ml is determined as follows:

$$\frac{\text{No. of fields in a 1 ml counting cell 1 mm deep}}{\text{No. of fields examined}} \times \frac{\text{Ml. of final concentrate}}{\text{Ml. of water concentrated}} = \text{The Factor}$$

For the direct count, all that is necessary is to know the area covered by the ocular, the number of fields in the counting cell, and the number of fields examined. Thus,

$$\frac{\text{No. of fields in the counting cell}}{\text{No. of fields examined}} = \text{The Factor}$$

Assuming that you have a one ml Sedgwick-Rafter counting cell (50 x 20 mm) and that you are using a 10 X ocular calibrated so that one field is equivalent to 1 square mil-

limeter, you would then have 1000 ocular fields in the total area of the counting cell. Therefore, if you counted 100 fields the factor would be

$$\frac{1000}{100} = 10 \text{ or the Factor}$$

If your check sheet shows a total of 67 organisms encountered in traversing 100 fields, the total number of organisms per ml of original water would be 670. Varying the size of the counting cell or the number of fields counted would only alter the mathematics involved. It is strongly recommended that the factor be maintained below 10, preferably at 5 or less. It could be accomplished in the example by simply counting 200 instead of 100 fields.

Precision of Counting Methods

The variation in successive counts in the same cell is considerable, even with the standard or approved methods, e.g. Sedgwick-Rafter and Foerst centrifuge. Littleford, et al. (1940) reported variations (table 1) on ten successive Sedgwick-Rafter concentrated counts when varying the areas or fields examined from 10 to 50.

It should be noted that the mean percent deviation in the ten individual counts from a concentrated sample, when 10 areas were counted, was approximately 20 percent with a maximum of 46 percent. By increasing the fields counted from 10 to 50, the mean percent deviation was decreased to approximately 6 percent.

At a rather low population density, the variation in a series of ten individual direct counts on the same one ml quantity of water determined by traversing 100 fields for each count was as follows:

Table 2

Order of Counts	Calculated No. of organisms per ml	Percent Deviation from the mean
1.....	550	12
2.....	410	36
3.....	470	26
4.....	960	51
5.....	790	24
6.....	580	9
7.....	670	5
8.....	530	17
9.....	820	29
10.....	580	9
Mean..	636	22

The mean percent deviation was found to be 22 with a maximum of 51 percent as a possible variation in the ten counts.

With a relative high population density (8000 per ml), ten strip counts across a 50 mm counting cell showed the following variation in actual number of organisms encountered:

Table 3

Order of Counts	No. of Organisms encountered in 50 mm	Percent Deviation from the mean
1.....	333	17
2.....	428	7
3.....	385	4
4.....	439	10
5.....	383	4
6.....	429	7
7.....	481	20
8.....	415	3
9.....	378	6
10.....	341	15
Mean....	401	9

The fact that with a standard method it is possible to get as much as a 46 percent deviation from the mean in its least accurate range would seem to indicate that the direct count with a 51 percent devi-

TABLE 4—COMPARISON OF PLANKTON COUNTS OF LAKE MICHIGAN WATER MADE BY THE DIRECT COUNT AND THE FOERST CENTRIFUGE METHODS AT CHICAGO, ILLINOIS, DURING SEPTEMBER, OCTOBER, AND NOVEMBER, 1946.

Month	No. of Samples	Average Number of Plankton per ml		Difference	Percent Deviation from the centrifuge
		Direct count	Foerst centrifuge		
September	50	841	810	31	4
October	29	974	916	58	6
November	29	1268	1179	89	8
Mean	36	1029	972	57	6

ation in its least accurate range might bear consideration and experimentation.

DIRECT COUNT VS. FOERST CENTRIFUGE

The first series of comparative counts was made on Lake Michigan water during September, October, and November of 1946. A total of 108 samples was examined for plankton organisms by both the direct count and the Foerst centrifuge methods. An approximately one liter lake water sample was collected and one ml was removed and immediately examined by the direct count. Organisms occurring in 100 fields were enumerated. As the control, one liter of the sample was concentrated to 10 ml by the Foerst centrifuge. One ml of the concentrate was then placed in the counting cell and the organisms in ten fields were enumerated. It is recognized that ten observations are usually considered the minimum when examining a concentrated sample and that the accuracy of the count can be increased by counting more fields. However, 100 fields are likewise considered the minimum allowable for the direct count; therefore, both methods were operating in their least accurate range.

The results show (table 4) that the direct count yielded a slightly higher mean for each month of the study. The percent deviation of the direct count from the centrifuge results increased with the population density from 4 to 8 percent. The direct counts show a mean deviation from the centrifuge results of only 6 percent during the entire three months of the study. It should be emphasized that this difference is the same as found for the Sedgwick-Rafter method when ten successive counts of 50 areas each were made on the same 1 ml of sample (Littleford, 1940).

DAILY FLUCTUATIONS IN PLANKTON POPULATIONS

Monthly averages as expressed in table 4 might leave the impression that the direct count always yielded higher counts than the Foerst centrifuge. Such is not the case. A casual observance of the daily counts found by each method (table 5) reveals what appears to be considerable variation from day to day and likewise between methods.

The problem of marked fluctuations in quantity of plankton from day to day is not new. It is evident in the data presented in most papers on biological productivity. It has been recognized that some of the difference is justifiably due to the precision of the method used. However, the marked similarities of the two curves representing population densities by two different methods seem to indicate that the organisms fluctuate much more than the methods of counting employed (fig. 1). If trends in population densities are the significant points to note, then it would appear that either the Foerst centrifuge or the direct count would serve the purpose equally well.

TABLE 5.—COMPARISON OF PLANKTON COUNTS OF LAKE MICHIGAN WATER MADE BY THE DIRECT FIELD COUNT AND THE FOERST CENTRIFUGE METHODS AT CHICAGO, ILLINOIS, DURING OCTOBER, 1946.

Date	Direct	Centrifuged
10-1-46.....	1455	996
10-2-46.....	2305	1444
10-3-46.....	1865	984
10-4-46.....	600	874
10-5-46.....	580	796
10-6-46.....	625	532
10-7-46.....	395	390
10-8-46.....	1405	1380
10-9-46.....	1110	1384
10-10-46.....	1265	936
10-11-46.....	595	638
10-12-46.....	515	534
10-13-46.....	620	698
10-14-46.....	530	620
10-15-46.....	1060	1014
10-16-46.....	680	554
10-17-46.....	1130	1160
10-20-46.....	755	956
10-21-46.....	1185	1108
10-22-46.....	1190	1342
10-23-46.....	1450	1090
10-24-46.....	1265	844
10-25-46.....	1345	934
10-26-46.....	785	690
10-27-46.....	470	690
10-28-46.....	620	948
10-29-46.....	1040	1272
10-30-46.....	360	814
10-31-46.....	1060	928
Total....	28260	26550
Mean.....	974	916

SAMPLE COLLECTING

Verduin (in press) has recognized the inadequacy of the "station method" of sampling in Lake Erie because of the marked fluctuations in the daily counts. He has recommended the use of mobile collecting points which are traversed by boat over rather extensive areas of the lake. By so doing, the migration of the plankton populations can be followed and significance is then attached to the marked day-to-day fluctuations at any station.

If one were so fortunate as to have unlimited funds, the ideal method of sampling a lake or large body of water regularly would be by helicopter. Little time would be lost in moving from point to point and thus an even more accurate representation of plankton population waves or accumulations in specific areas could be established.

It seems quite evident that the method of collecting the sample for examination is probably more important than the selection of a method of counting, if we are to smooth out the daily fluctuations so often reported in plankton studies.

DIRECT COUNT VS. SEDGWICK-RAFTER METHOD

Beginning March 17, 1947, and continuing through April 15, 1947, plankton samples were collected daily from the raw Lake Michigan water, the chemically treated water, the settled or filtered water and from

TABLE 6.—DIRECT COUNT AND SEDGWICK-RAFTER METHOD COUNT ON WATER OF VARYING POPULATION DENSITIES IN THE SOUTH DISTRICT FILTRATION PLANT OF CHICAGO FROM MARCH 17, THROUGH APRIL 15, 1947.

Kind of Water	Sampling days	Average Plankton No. per ml		Difference
		Direct	Sedgwick-Rafter	
Raw water (untreated)	30	614	414	200
Chemically treated	30	97	35	62
Settled (filtered)	30	11	3	8
Outlet	30	15	6	9

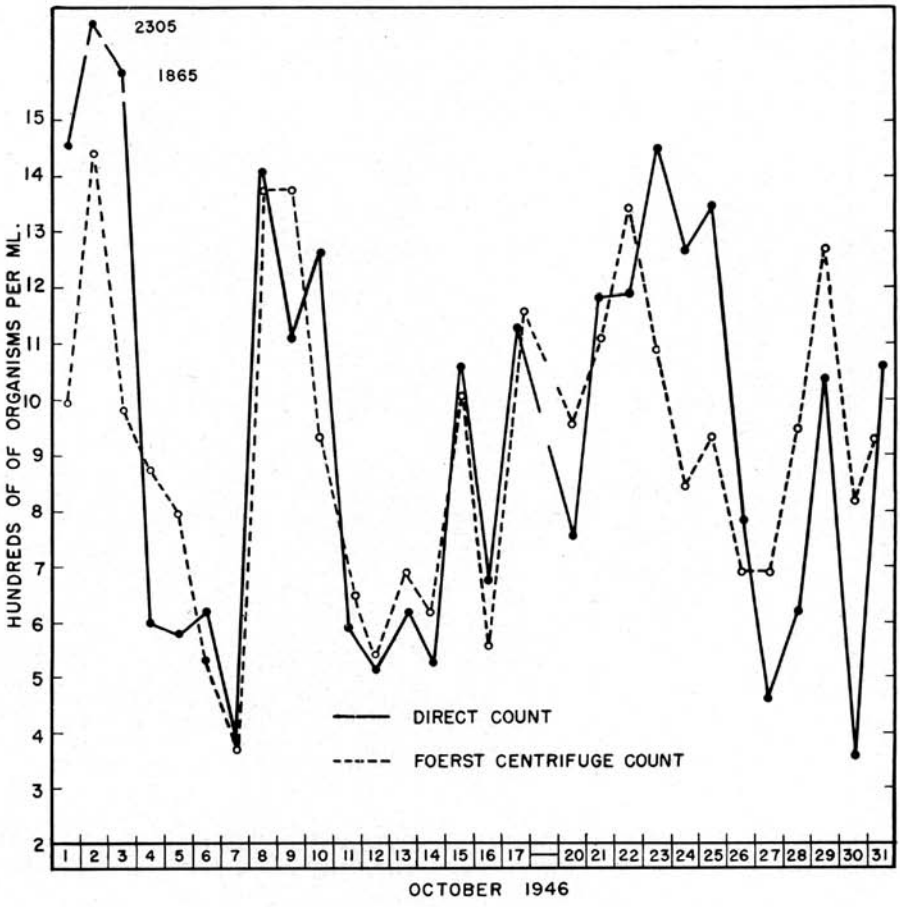

FIG. 1. Comparative plankton counts made by the Foerst centrifuge and the direct count methods on untreated or raw Lake Michigan water during October 1946.

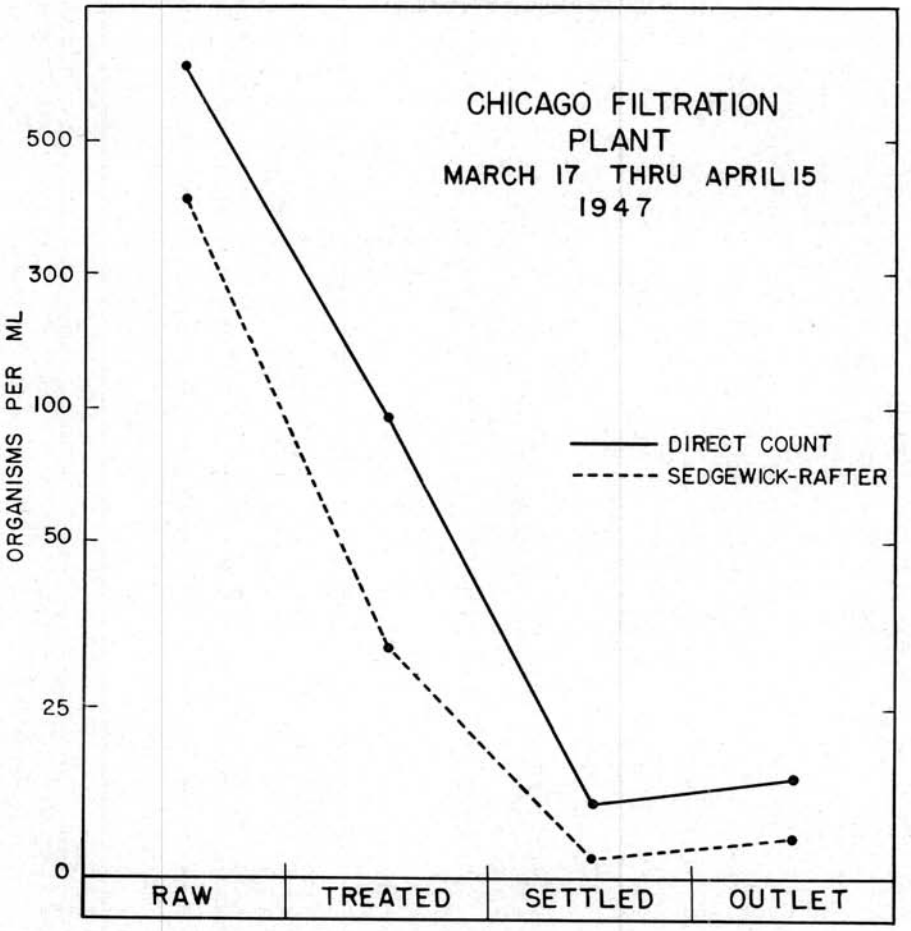

FIG. 2. Comparative plankton counts made by the Sedgwick-Rafter and the direct count methods on water of three different population densities.
Raw—Untreated Lake Michigan water.
Treated—Chemically treated and partially settled water.
Settled—Settled and filtered water.
Outlet—Mixture of treated and settled water.

the outlet water of the Chicago filtration plant. Both the direct count and the Sedgwick-Rafter methods were used on the same common sample. By using the raw, treated, and settled water, the two methods were compared at three quite different population densities. The outlet water during the study was a mixture of treated and settled water.

The direct count yielded a considerably higher average plankton number in all of the population densities (table 6). Yet both curves (fig. 2) followed the same general trend. Which method is nearer to the actual number of organisms present is not of greatest concern. It is important that both methods are consistent. It would appear that for a routine record of the efficiency of plankton removal in a water

plant, the direct count is as reliable as the Sedgwick-Rafter method and is much less time-consuming. For taxonomic or qualitative work on species, a tow net sample or some other means of concentration would always be recommended over the direct count method.

Summary

A simplified method of counting plankton organisms by direct observation without concentration has been described. In routine microscopic examinations over extended periods of time, the results by the direct count have been compared with the Sedgwick-Rafter and the Foerst centrifuge methods.

The data presented seem to indicate that for simplicity, economy, consistency, and convenience the direct count without concentration of the sample is highly satisfactory and often desired over other more involved and expensive methods.

Acknowledgments

The writer is indebted to the Department of Public Works of the City of Chicago where most of the experimental data was collected while he was an employee of the Water Purification Division. Oscar Hewitt is Commissioner of Public Works; W. W. DeBerard is City Engineer; John R. Baylis, Engineer of Water Purification, and H. H. Gerstein, Chief Filtration Chemist at the South District Filtration Plant where much of the laboratory work was completed.

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