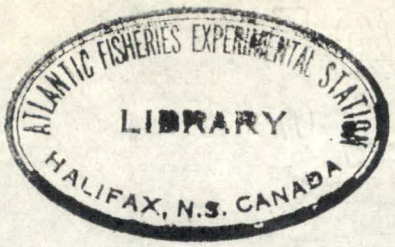

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STANDARDIZATION OF PLANKTON APPARATUS

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STANDARDIZATION OF PLANKTON APPARATUS

by

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At the third Pan-Pacific Science Congress in Tokyo, 1926, Dr. Wilbert A Clemens, Director of the Pacific Biological Station, Nanaimo, B.C., submitted a paper with the title: Suggestions as to the Standardization of Plankton Methods. In this paper Dr. Clemens suggests that a committee composed of representatives of the various countries might develop a program and: "2. Recommend standard methods of procedure, that is, might describe in detail the kinds of nets which should be used, the methods to be used in collecting samples, and the procedure to be followed in enumerating etc."

Some of these suggestions had already been carried out. The lack of uniformity in the international investigations being felt very badly, the Counseil Permanent Internationl pour l'Exploration de la Mer, at the council meeting held in July 1921, passed a resolution regarding a standard net for quantitiative plankton investigations. An exact specification of this net was to be furnished by Mr. Nelson and circulated through the bureau. However, the specification had not appeared prior to the next meeting of the council, in 1922, and there was then passed a further resolution which among other items, changed the length of the filtering surface of the net from three to two meter,- in the interest of economy. A more important statement in this resolution is, that the nets shall be manufactured of silk similar to that made by Messrs. Albert Wudler of Zurick, Switzerland.

Fine mesh No. 25, 77 strands in 10 mm.

Coarse mesh No. 3, 23 strands in 10 mm.

A complete specification of the nets was promised from Mr. Nelson's hand at an

early date. Owing to the death of Mr. Nelson the specification was never completed, and first after the council meeting in Paris in October, 1923 there is really done some work on it which results in the Publications de Circonstance No. 84 (C.H. Ostenfeld and P. Jespersen. Standard Net for Plankton Collections) published in Copehhagen in August, 1924. This publication contains descriptions of standard nets, one for vertical hauls and another for horizontal hauls, besides there is a description of a method for the vertical haul. This is really the most important part of the pamphlet, as it is quite obviously that this method overcomes four of the five chief objections to vertical townets viz: 1. The effects of the rolling of the ship, which in heavy seas can be so severe that they may burst the net. 2. The irregularity of the speed of hauling, except in the case where the net has been clogged with Phaeocystis or like organisms, but this exception in itself overcomes No. 3 objection, as it gives a mean by which to tell when the net is clogged and thus notifies the investigator that this sample cannot be considered a true quantitative representation of that station. 4. When the net is clogged it is sometimes hauled out full of water, a frequent cause of bursting.

This method was first described by the inventor N.J. Buchanan-Wollaston in his: Report of the Spawning-Grounds of the Plaice in the North Sea. (Board of Agriculture and Fisheries. Fishery Investigation's Series II, Vol. II, No. 4, 1914). The same description is later quoted in extenso in the mentioned Publication de Circonstance No. 84, wherefore I shall not here go into further detail.

The Counseil permanent international had thus done its share towards the possibility of comparing quantitative plankton investigations performed in different places by different investigators, but they did not touch the problem whether the plankton net in itself was sufficiently efficient to be completely

reliable.

One should think that the excellent works of Hensen, Lohmann, Apstein and several others had dealt with this problem to such a degree that it might be possible to calculate the exact number of plankton organisms in any volume of water from any depths just by counting a few samples and then for the rest use some tables, but in spite of all the mathematics these investigators succeeded in putting into the plankton methods it is still a very uncertain task to compute the number of organisms in relation to the volume of water. As a matter of fact, so uncertain that, unless the numbers show remarkably big differences, no investigator dare build anything definite upon such figures. Had Hensen had the object of finding what factors influence the number of Coccolithophora, instead of calculating the number of fish eggs in the North Sea, it is possible that with his great skill as an investigator and mathematician he would have found the way through all the obstacles the modern quantitative plankton investigation is fighting against. The most important of which is the uncertainty in regard to the efficiency of the net used.

Hensen gives, in his "Methodik der Untersuchungen bei der Plankton Expedition" a theory for the efficiency of nets, which he comments further upon in his following works (Das Leben im Ozean nach Zahlungen seiner Bewohner; Ueber die quantitative Bestimmung der kleineren Planktonorganismen und ueber den Diagonalzug mittelst geeignerter Netzformen; Nachtrag zu der Arbeit ueber die quantitative Untersuchung der kleineren Planktonorganismen; and Zur Feststellung der Unregelmassigkeiten in der Verteilung der Planktonten. First in the last one, published in 1912 seventeen years after he had started his plankton investigations, does he begin to see that the equal distribution of the plankton which he at first so gladly had presumed did not exist at all.

Also Lohmann produces his big works on the same subject; Ueber des Fischen mit Netzen aus Mullergaze No. 20 zu dem Zwecke quantitativer Untersuchungen der Auftriebs, and Neue Untersuchungen ueber den Reichtum des Meeres an Plankton und ueber die Brauchbarkeit der verschiedenen Fangmetoden. Especially the latter has a very good discussion on the pro et contras for the different methods of obtaining plankton samples. Noteworthy is that he herein shows that the sources of error which C.A. Kofoid objected so much to in his "Note in Science (N.S. Vol. VI, pp. 829-832. On some important sources of error in the Plankton Method), also apply to marine plankton, wherefore he uses a pump to obtain known volumes of water from known depths.

This method which first was introduced by C.A. Kofoid (Plankton Studies. I. Methods and apparatus in use at the Biological Experiment Station of the University of Illinois, Bull. Illinois State Lab. Nat. Hist. Vol. V), has many advantages, but for use in deeper water it is rather prohibitive as the handling of such a long hose is very difficult. The main reason for introducing this method was the wish to obtain quantitative samples of the nanoplankton, that part of the plankton that will pass through the meshes of an ordinary plankton net. The question itself: How to get all the plankton present in a certain volume of water, has been discussed over and over again in the literature. Of course it is of utmost importance when dealing with the total productivity of the ocean, but the modern plankton investigation is more interested in the relation between certain species of plankton organisms and their environmental factors, such as salinity, concentration of nirate, silica etc., and the relative small amount of nanoplankton lost through the nets may therefore be considered of less importance.

The problems of the plankton methods as regards the Pacific has lately

been dealt with by a number of investigators. Most of the work has been done by the Scripps Institution of the University of California. At the third Pan Pacific Science Congress in 1926 Professor W.E. Allen of this institution gave a paper on the Investigations on the Phytoplankton in the Pacific Ocean in which he very thoroughly discussed the work hitherto done, the methods used for it, and lastly the results, especially as foundations for further work. As a whole this summers experiments make me agree with his opinions except regarding the preservation of plankton samples for quantitative work, which point I shall deal with more in detail at that part of the report.

The latest work by the same author is to be found in the Bulletin of the Scripps Institution of Oceanography, La Jolla, California. Technical Series Vol. 2, No. ' (Berkeley 1930) "Methods in quantitative research on Marine Microplankton". It is a comparison of series of catches treated after different manners, and his conclusions confirm the results of several other investigators. It is, however, regrettable that he does not give a more reserved statement in the first part of his conclusion: "The study of these series shows positively that no method of rapid filtration (such as that through No. 25 silk) can give dependable results in quantitative investigations of the smaller diatoms and dinoflagellates, especially in periods of slight abundance". It is right that so far no method has been found, but it is my belief that it would be possible to design a centrifuge for that work with just as high an efficiency as the centrifuges now so commonly in use for milk-separating or clarifying. The main difference is that in this case it is the sediment that is the important part. Therefore, the type of centrifuges already tried out for this purpose has been entirely unsuited for this kind of work. Their great number of discs and plates spread the sediment over such a big and, for the main part, dif-

ficultly accessible surface that, although even one hundred per cent may be detained in the separator bowl, it is very questionable how many per cent the investigator will be able to recover from the bowl.

Chancey Juday in his: "A third report on Limnological Apparatus" Transactions of the Wisconsin Academy of Sciences, Arts, and Letters, Vol. XXII, 1926, gives a description of a small centrifuge with no discs at all. The capacity of it is undoubtedly too small to be of any value for marine work, but its bowl recalls to me the bowl of the sharpless separator, and my opinion is that there may lay an solution of the problem in this peculiar discless type of separator.

The experiments during the summer of 1931 fell in three parts.

1. Comparison between plankton net and a plankton bucket in order to find the efficiency of the net.
2. Comparison between the common centrifuge method for measuring the volume of plankton and the displacement method as developed by Dr. I.P. Jacobsen.
3. A study of the influence of different preservatives on the same sample of plankton.

1. Comparison between plankton net and plankton bucket

The net used was a closing net of the type described by C. Juday in "Limnological Apparatus" (Transactions of the Wisconsin Academy of Sciences, Arts, and Letters, Vol. XVIII, PartII). The dimensions of it were as follows. Diameter of small ring 30 cm., of large ring 45 cm., length of side of the sifting cone 84 cm. Total sifting area when account is taken of the seams and the four windows in the bucket 5466 square centimeters. Area of opening 706.85 square cm. Sifting material Bolting Silk No. 25.

The plankton bucket used was the one constructed after the design of Mr. L.L. Bolton. The capacity of it was ten imperial gallons.

Due to some necessary alterations in the closing mechanism of the bucket it was impossible to obtain the desired samples until shortly before I had to leave for the University. However, I succeeded in getting four good series from one station, taken as soon after each other as the circumstance, that the same winch had to be used for both net and bucket, would allow. The weather was rather rough which forced us to take the series just outside the Dodd's Narrows instead of, as intended, at Station 35.

The first series was taken with the net, with the ordinary routine for plankton samples for quantitative purposes. First from 50 - 30 meter, then from 30 - 20, 20-10, 10-6, 6-4, 4-2, 2-1, and from 1 meter to the surface. The second series was taken with the bucket, which was lowered to the depth of one meter, two meters, and so on, a sample from each meter until the sample from ten meters showed such a little amount of plankton that it was obvious that it would be impossible to measure the volume of it. The water from the bucket was sifted through a small net of Bolting Silk No. 25, and the plankton immediately preserved by adding 10 cc of B.C. plankton preservative. The third series was also taken with the bucket and as soon as possible after the second. The fourth series was taken with the net.

The volume of the thus obtained samples were standardised to 168 cc and 15 cc of each centrifuged until the volume of plankton showed to be constant. The results of this volume determination are given in the following table.

	s	1	2	3	4	5	6	7	8	9	10 metres
											$\frac{cc}{10}$
Ser. I		6.2	3.5		2.5		2.5			3.0	
Ser. II	2.5	2.0	2.0	1.0	1.6	1.3	1.0	1.0	.8	?	?
Ser. III	2.5	2.0	1.8	1.0	1.8	1.3	.9	?	?	?	?
Ser. IV		7.5	4.5		3.5		3.0			2.5	

If we out of these figures compute the volume of plankton in one cubic meter of water we get the following results

Cubic centimeters of plankton in one cubic meter of water.

Depth	Ser. I	Ser. II	Ser. III	Ser. IV
1-s	9.824	54.399	54.399	11.883
2-1	5.546	49.339	46.761	7.130
4-2	1.981	37.823	37.823	2.773
6-4	1.981	32.070	32.892	2.376
10-6	1.188	?	?	0.968

These figures show the average plankton content for the given depth. For the series taken with the net the volume of the water is taken as the length of the hawl times the area of the net opening. Comparison of the figures for the net with the corresponding figures for the bucket, the volume of which is known with certainty to be ten gallons, shows that the efficiency of the net is very variable. It goes as high as ca. 18% but this seems to be the exception and the average is somewhere around 10%.

Hensen's net coefficient when calculated for the same net came to ca. 0.31, which is in conformity with what other investigators have calculated for nets of similar dimensions but certainly not with the actual results. These, on the other hand, coincide very well with the results obtained by C.A. Kofoid as published in his "On some important Sources of Errors in the Plankton Method)(Science N.S. Vol. VI, pp. 829-832; 1897).

These experiments are not the first experiments with plankton buckets done for the Biological Board of Canada. Dr. Bajkov used for his Jasper Park lakes investigations a self-closing bucket of a somewhat different design, which he describes in "Reports on the Jasper Park Lakes investigations 1925-26 VII. A study of the plankton. (Contributions to Canadian Biology and Fisheries. New Ser. Vol. IV, 1929). It could have been desirable to compare this type of plankton bucket with the one of Mr. Bolton's design, but owing to Dr. Bajkov's absence from Winnipeg it has been impossible for me to obtain a more detailed description in time for this report.

2. Comparison between the common centrifuge method of measuring the volume of plankton and the displacement method as developed by Dr. I.P. Jacobsen.

While waiting for the plankton bucket to get into perfect working order I determined the volume of plankton in a great number of samples in order to obtain a certain routine in this procedure, so as to ascertain that no error due to the handling of the centrifuge should spoil the experiments. During this work I found that in many cases it was impossible to read the volume of the plankton as it was less than the smallest division on the graduates used for the centrifuging, these being calibrated only into one tenth of a cc and the amount of plankton in fifteen cc of sample very often being far less than

that. It lay therefore near to think of the possibility of another method for measuring of the plankton volume and, when I in my perusing of the literature on plankton methods came upon I.P. Jacobsen and Ove Paulsen: A new apparatus for measuring the volume of plankton samples by displacement. (Meddelelserfra Kommisionen for Havundersgelser. Serie Plankton. Bind I, No. 11, Copenhagen 1912). I found it necessary to try this method in order to make sure whether it was better than the usual method or not. At first I assembled an apparatus as described by Dr. I.P. Jacobsen, but I found that it was not quite as practical as it could be, and, more important, that the many rubber connections were a bad source of error which should be avoided. I therefore fused all connections and at the same time added a simple device for refilling the apparatus and thus speed the procedure somewhat up. The new version of the apparatus is shown in the figure.

The principle of the measuring by this apparatus is: A hardened paper filter is placed in the funnel and the stopcocks are turned so as to allow the measuring liquid to flow into the funnel and fill it right up till the opening of the spout, which is on the highest edge of the funnel, just when the liquid is about to overflow the stopcock at the funnel is closed, and the point where the surface of the liquid stops in the measuring tube is noted. The apparatus is then refilled, while the liquid is drawn off from the filter by means of the suction pump. This is repeated several times until the point where the liquid stops is constant. This poins is then the zero point for that filter. Now a certain amount of a sample, already made up to the standard volume, is filtered through the filter, the plankton will remain in the filter and when the measuring liquid again is allowed to flow into the funnel and again stopped just when it is about to flow over, it will this time have a higher level in the measuring tube. The difference between the zero point and this new level equals

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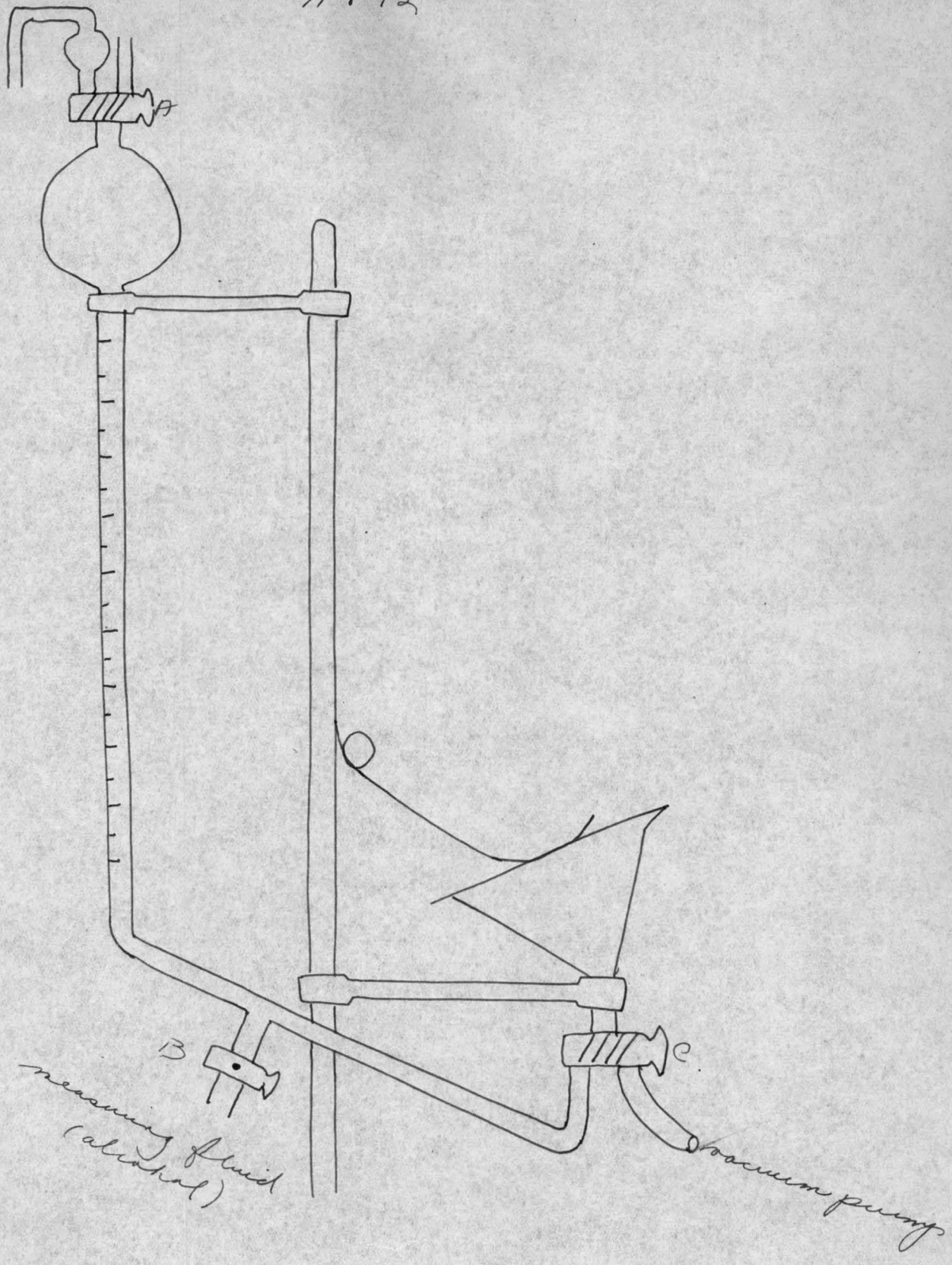

the volume of the plankton. For the determination of the zero point the apparatus was found to work with an exactness of one one hundredth of one cc., but with the plankton in the filter it was often difficult to reach the same point of dryness, and the error in this case is most likely twice as big. The adhesion of the measuring liquid to the walls of the bulb and the measuring tube makes it necessary to leave the apparatus for about five minutes before reading.

At first distilled water was used as the measuring liquid, but it proved that it was nearly impossible to get rid of all the air bubbles which would be in the plankton, wherefore it was necessary to change to alcohol. Dr. I.P. Jacobsen records the same experience from his work with the apparatus.

It is possible to use the same filter for three or four samples either, if the plankton is to be weighed also, by rinsing the filter very carefully for each determination or, if there is no more use for the sample, just by taking the new reading as the zero point for the next determination. When a filter has been used for some time it becomes too close to be used with advantage, as the filtration takes too long time.

The accompanying five graphs show the relations of the volumes found by this method to those found by the ordinary centrifuge method.

In the four of the series also the weight of the plankton was determined after drying to constant weight at a temperature of 100 to 105 degrees centigrade.

Several more series were measured, but in many cases it was found that the plankton content was too poor to be observed with any degree of exactness; a number of the samples were measured before it was found out how severe the errors due to the rubber connections were. After it was found out, these, of

course, could not be used for comparison.

Considering the time involved in the zeropoint determination and the inexactness that necessarily always will be due to the liquid retained in the plankton by capillary attraction, it is very doubtful whether this method has any advantages at all to the ordinary method. The amount of fluid retained by the capillary attraction will vary with the type of plankton and a sample of Chaetoceras will therefore seem larger than a sample of Coscinodiscus even if they are of exactly the same volume. The same is the case in the centrifuge method as the long spines will prevent the Chaetoceras from being packed as closely together as the Coscinodiscus.

3. A study of the influence of different preservative on the same sample of plankton.

The preservation of the sample is a part of the plankton problem that by most of the authors dealing with plankton methods seems to be regarded as rather unimportant. A few lines, if anything at all, is all they give to it. Naturally it would be the ideal to study the living sample, but the great number of samples necessary for a series from a single station eliminates this possibility.

H.H. Gran gives a promising title for his Publication de Circonstance No. 62. "Preservation of samples and quantitative determination of the plankton", but the content of this publication is only a mentioning of the fact that for organisms with a skeleton of calcium carbonate as e.g. Coccolithophoridae it is no good to use a preservative containing any acid, as this dissolves the skeleton, but nevertheless he has found that Flemmings Solution was an excellent preservative and very much to be recommended. The rest of the work is an enumeration of the organisms found in a number of samples.

Prof. W.E. Allen in his paper on Investigations on Phytoplankton in the Pacific Ocean just mentions that: "Formaldehyde is the best preservative for general purposes both for convenience and excellence of preservation.

However, formaldehyde has exactly the same bad effect on lime particles as acids have, it dissolves them. Besides the excellence of preservation, sometimes, is not quite as excellent as it might be wanted. The tendency for polymerisation in formaldehyde may often result in a solution much weaker than expected and a correspondingly poorer preservation. The solution of lime particles can be prevented to a certain extent by adding some weak neutralizer as e.g. sodium borate but the weakening is hard to check even in the darkness.

For most of the Phytoplankton it has been found that the B.C. Fixative is an excellent preservative. The organisms are found to be in a very good state of preservation. During my work on volume determination it occurred to me, however, that the fixative contains a high percent of alcohol, which possibly might expell water from the organisms and thus cause a change of the volume. In order to get a little light on this problem I collected a big sample of surface plankton, measured first the volume of plankton in the living sample, divided the rest into three equal parts and fixed one with common formaddehyde, the second with Flemming's solution, and the third with B.C. Fixative, being careful that the same volume of fixative was used for each part of the sample. Immediately after the fixation the volume was again determined, the results were as follows:

	living	Formol	Fixed by Flemming's	B.C.
cc. of plankton in 15 cc	2.37	2.0	1.8	1.8

The actual figure found for the living plankton was 2.8, but as addition of the fixatives reduced the plankton concentration in the samples I have reduced this figure for the living sample accordingly.

The samples were then left for a time, approximately three weeks, and the volume then again determined. The result this time made me believe that I had failed to shake the samples thoroughly enough after the long standing, wherefore I used all the rest of the samples, exactly 75 cc or 5 x 15 for each. The results were:

	Volume in cc of plankton fixed by				
Formalin	1.0	1.0	1.1	0.9	0.9
Flemming	1.5	1.5	1.5	1.5	1.3
B. C.	1.5	1.5	1.5	1.5	1.5

Another sample treated in the same way did not show such a bad result:

	Formalin	Flemming	B. C.
Volume fresh fixed	2.4	2.3	2.25
after three weeks	2.2	2.0	1.9

Altogether this experiment shows that there is here a problem which must be considered in the future quantitative plankton investigations.

CONCLUSIONS.

1. The experimental determination of the efficiency of plankton net shows that the net can hardly be relied upon for any quantitative investigations of the microplankton.

2. The common method of determining the volume of plankton by means of settling in graduated tubes exposed to centrifugal force is to be preferred to the much more elaborate method by displacement.

3. As the common centrifuge method is now it is not always it is satisfactory. Very likely this could be helped by using the graduates especially adapted for bacteriological purpose, with the one cc at the bottom

divided into one one hundredth of one cc. for samples where the plankton is less abundant.

4. There is a considerable problem in the shrinkage of the volume of the plankton during fixation by different fixating agents. This problem ought to be solved as soon as possible.