A COMPARISON OF PLANKTON COUNTS FROM THE TRAP-NET AND WATER BOTTLE CENTRIFUGE TECHNIQUES

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INTRODUCTION

Quantitative plankton methods have been the subject of considerable study, but many workers making routine plankton counts have concerned themselves little with problems arising out of certain common techniques.

In a study of collections with the Birge quantitative, closing, plankton tow net (Kraatz, 1931), the writer made counts under binocular in the entire Sedgwick-Rafter counting cell, not only of zooplankton such as Entomostraca and Rotifera, but also of as many others of somewhat smaller size as feasible; and as usual, counted the smaller plankters on microscope (16 mm. objective and 7.5 ocular) with Whipple ocular micrometer in 20 squares, multiplied by 50 to get the cell counts. For plankters counted both ways, where numbers involved were small, the numbers secured by multiplying were almost invariably larger, often much larger than the whole cell counts, which were correct. One can conclude that the larger the portion counted of any kind of plankter in the cell, the more accurate the results.

In the present paper while the same comparison is in the background, the principal comparison is between counts of organisms collected by the trap and the water bottle.

The main objectives of the writer's investigation, the seasonal and other plankton distribution, will be presented in another paper, where also will be given records of temperatures and chemical tests. There also acknowledgments will be made to all those who assisted in the collection and otherwise.

Realizing after studies were made that statistical checks on apparent discrepancies of trap-net and water bottle centrifuge counts would be important, the writer entirely uninformed in such matters, secured the aid of Dr. Wm. E. Ricker, who gave most valuable information in personal communications and in his papers (Ricker 1937, 1937 (a), 1938) to which readers should refer for explanation of statistical methods in plankton work.

COLLECTING METHODS

Collections were made in Turkeyfoot Lake, near Akron, Ohio, from August, 1936, to March, 1939, with unavoidable omission of certain winter months. The Foerst plankton trap, 10 liters capacity, having cone net and bucket of No. 25 silk bolting cloth, as on the Birge net and the Kemmerer-Foerst brass water bottle, 2 liters capacity, both donated by the Ohio Division of Conservation, were used in collecting.

Trap samples, 10 liters, were uniformly concentrated to vial samples of not quite 40 c. c. Water bottle samples were uniformly one liter; the other liter was used for chemical tests. The one liter used was thoroughly representative of the two liters collected.

DISCUSSION OF COLLECTING METHODS

The trap-net is better than the net alone, in that it obviously collects a known quantity of water, but is subject to the same losses of small organisms through meshes of the concentrating net. The net used was old and had previously shrunk, (as new cloth will shrink in water) and the meshes were to some extent clogged.

Most water bottle plankton collections have been one or two liter samples. It is impracticable to carry a set of bulky ten liter or even five liter sample bottles. Larger samples would give theoretically better representation of lake population, a point considered negligible by many workers, especially when they count only the smallest plankton from the water bottle.

LABORATORY METHODS

Preservation of trap samples was accomplished at once by having about one c. c. of formaldehyde in the vial when collection was made. In the laboratory a little distilled water was added to make each trap sample exactly 40 c. c. later as the sample was to be examined. After thorough agitation one c. c. was put in the Sedgwick-Rafter counting cell.

The one liter water bottle samples were not preserved initially, but immediately after return to the laboratory they were centrifuged, each in about 7 minutes for one centrifuging, as advised by Foerst, and by Juday. The Foerst electrically run water centrifuge has an r. p. m. of 15,000. The plan of two centrifugings for each sample was adopted. Distilled water was added to the residue to wash it out of the cup of the centrifuge. Later for examination the samples were uniformly made up to 20 c. c.

In a great deal of other plankton work, counting cell samples from the trap-net were examined for so-called net plankton and from water bottle, for the so-called nannoplankton or dwarf plankton. In recent Ohio Conservation Division work trap samples were examined for zooplankton and water bottle samples for phytoplankton, which is efficient from the standpoint of two observers working with the separate samples and makes for a biological division of groups desired.

The writer decided to examine trap samples for all plankton and water bottle samples likewise, to secure a check if possible, comparing the two collecting and concentrating techniques. For both, the one

c. c. in the counting cell on low power binocular, × 48, with aid of mechanical stage, was counted completely for Entomostraca, Rotifera and also others like Ceratium, when feasible, and then the smaller more abundant forms were counted in 20 squares of the Whipple ocular micrometer, on the microscope with 16 mm. objective and 7.5 ocular. Therefore, a comparison of samples from trap and water bottle could be made.

Due to the particular size of the samples, the trapnet count, when of the whole cell as for larger plankters, represented ¼ of a liter, but when counted in 20 squares (multiplied by 50 to get the total) the actual count was of 1-200 of a liter. For the water bottle the two counts represented 1-20 of a liter and 1-1000 of a liter respectively.

DISCUSSION OF PLANKTER SIZE IN RELATION TO COLLECTION AND CONCENTRATION METHOD

It has long been recognized that even the finest mesh tow nets allow the smallest plankton organisms to escape and that if the plankters are very slender long colonies or individuals, they might be retained in variable degree, but could if exactly endwise pass through the net meshes. The organisms too small to be collected by net, the nannoplankton, are collected by water bottle.

But to count zooplankton only from trap samples and phytoplankton only from water bottle centrifuged samples, seems arbitrary, especially since many Protozoa would belong to nannoplankton, and on the other hand, many plant colonial types, larger Cyanophyceae and Chlorophyceae and some diatoms are net plankton.

A separation of net plankton and nannoplankton on basis of size is essential, but it does not seem feasible to maintain exact size difference as indicated by Welch (1935, p. 208). A separation on basis of kinds, species or genera, though not necessarily accurate, seems practicable.

In most routine work genera alone can be listed. It is notable that Birge and Juday (1920, p. 60) list in net plankton, besides Entomostraca and Rotifera, the following: Ceratium, Microcystis, Coelosphaerium, Aphanizomenon, Anabaena, Lyngbya, Staurastrum, Melosira, Tabellaria, Fragilaria, Asterionella, Stephanodiscus, but again among nannoplankton, p. 90, list Coelosphaerium, Stephanodiscus, fragments of Aphanizomenon, but also thirteen other genera of algae and Rhizopoda and Ciliates.

Turkeyfoot plankton includes prominently a large type of Coelosphaerium, large Anabaena, and also considerable Microcystis and abundant Aphanizomenon. The latter is a more slender filament than the Anabaena. All these, and also the diatoms Asterionella, Synedra, of large size, and Fragilaria, occurred prominently in trap-net samples showing very typical development and decline periods during a year. However, certain comparisons of net and centrifuged samples will be made later.

In contrast to the above which can be termed net plankton, there were found at certain times a very much smaller kind of Synedra, a very tiny filament identified as a small Oscillatoria, a smaller type of Coelosphaerium, Trachelomonas, and in just two collections one fall

a tiny diatom Amphora. Practically none of these were revealed in trap-net samples. They occurred in centrifuged samples, and whenever they did, especially the tiny Synedra, and Oscillatoria and Amphora, in enormous numbers.

But nannoplankton have not been revealed adequately in this study, despite water bottle collections, due to limitation of practicable magnifications. The Sedgwick-Rafter counting cell and Whipple micrometer permit only work with 16 mm. objective and 7.5 ocular. For identification occasionally a 12.5 ocular was substituted, but all counts made with 7.5 ocular. No examination was made of a drop mount under thin cover glass and high powers. Dr. C. E. Taft identified many smaller algae and Dr. L. E. Noland some small Protozoa. Dr. Noland pointed out the difficulty of identification of preserved ciliates and others. While these types were numerous, most did not occur in great numbers relatively and it was found impossible to consider and count them in the routine work. The total quantity of nannoplankton, however, would be enormous.

The nannoplankton can be omitted from this paper as the comparisons are essentially of net plankton, as revealed in the two different collecting and concentrating techniques.

LOSSES IN CENTRIFUGING

The centrifuging process tended to break up some larger filamentous colonies, especially Anabaena, making it harder to count the colonies. Asterionella maintained its star-shaped arrangement remarkably well, except in a few instances. A few others were sometimes broken, most surprisingly the hard-shelled Ceratium. Sometimes its points were broken; sometimes it was broken in two across at the groove.

A worse feature was the loss of some blue-green algae over the top of the small inside cup of the centrifuge so that they went with the overflow water instead of the sample. The large Coelosphaerium and the Anabaena were lost in large degree, Microcystis to nearly the same extent, Aphanocapsa, somewhat less and Aphanizomenon usually was not lost. This will be again brought out in the tables. Dr. Juday wrote me that he had little trouble of this sort in his long experience, except with Aphanizomenon.

The centrifuge was working well. A stroboscope test on the empty centrifuge showed not far below 15,000 r. p. m.

Coelosphaerium and Anabaena are low in specific gravity, especially when more or less in condition of water bloom, but less so in colder weather when numbers are low. They tend to gather in the top layer of water. In one case a liter bottle purposely left standing for some time before centrifuging, showed a thin line of these gathering on the water surface. In the case of the net condensed vial samples, a dense layer of from one to two mm. thick would form on the top of the four-inch column in the vial. At the same time other plankton tended to sink to the bottom. Even in counting cell samples, in a very short time after making the cell mount, these same blue-greens would tend to come up to the cover glass. To be sure, the net samples in the vials and all samples on the microscope were preserved, but the preservative

was a slight amount compared to the water volume. And it must be repeated that material in samples at centrifuging was alive.

Every sample was centrifuged twice. Nevertheless, as shown in the tables, nearly all these blue-greens were lost in centrifuging. More centrifuging was not feasible in routine work. But a test case was made in which one liter was centrifuged five times. Lengthy examination showed ample Coelosphaerium and Anabaena in overflow water, but none in the sample after the first centrifuging. Again in each of the subsequent centrifugings of the same overflow water, large but decreasing numbers were indicated in the overflow water, and but slight increases in the successive samples, but a fair improvement in the last. It is surprising that there should not have been more improvement, especially since all other organisms were successfully removed the first time. A trap-net sample taken at the same time and place as the above centrifuged sample, proved a rich supply of these blue-greens present.

REPRESENTATIVE PLANKTON COMPARISONS

DISCUSSION OF TABLES

In the tables the most direct comparison is between counts of plankton collected by trap and collected by water bottle, the method of concentration being by net as compared with centrifuge, indicated by "net" and "centr." respectively.

TABLE I

CYANOPHYCEAE

Numbers in 1 Liter of Turkeyfoot Lake Water, June 19, 1937

ORGANISMS	SURFACE WATER		4 METERS		8 METERS		12 METERS	
	Net	Centr.	Net	Centr.	Net	Centr.	Net	Centr.
Coelosphaerium	4,100	460	5,200	1,320	1,400	180	896	1,520
Aphanocapsa								
Microcystis	800		600	380	400	60	236	140
Oscillatoria								
Anabaena	8,000		6,800		1,400		1,272	
Aphanizomenon	153,600	337,000	183,200	391,000	25,200	8,000	37,000	150,000

Tables II and III. All main groups, represented by the chief plankton organisms found more or less throughout the collecting period, are included. Of these the green algae were least common. Many other kinds were found from time to time. All types included are net plankton, though some are not so perfectly collected by net.

Certain nannoplankton were counted, but as previously explained, they are omitted as being outside the scope of this paper.

Cyanophyceae.—Tables I, II and III. A glaring lack in centrifuged samples (as described above) of Coelosphaerium, Anabaena, and the less common Microcystis is seen. This applies less fully to the Aphanocapsa.

TABLE II

CYANOPHYCEAE AND OTHERS

Numbers in 1 Liter of Turkeyfoot Lake Water, July 19, 1937

ORGANISMS	SURFACE WATER		4 METERS		8 METERS		12 METERS	
	Net	Centr.	Net	Centr.	Net	Centr.	Net	Centr.
Coleosphaerium	4,000		4,400	1,450	2,200		4,400	75
Aphanocapsa	1,400		1,200		200		1,200	
Microcystis	3,600		2,400	1,250	1,400		2,400	
Oscillatoria				1,250			200	
Anabaena	65,600	5,000	49,607	7,500	10,400		42,800	
Aphanizomenon	18,400	232,000	34,400	46,250	4,800	2,500	20,400	
Melosira	1,000	3,000	800	1,250	1,800	5,000		1,250
Synedra								
Asterionella								
Fragilaria	800	3,000	600	5,000			200	1,250
Stephanodiscus	1,200	1,000	1,000		2,400		3,000	
Staurastrum	36		40	150	80		100	
Pediastrum	20	40				75	24	
Ceratium	4,940	760	2,964	175	644	150	656	50
Polyarthra	492	540	144	150	12			
Keratella	324	460	656	425	192	325		
Synchaeta								
Asplanchna								
Notholca	92	100	12	75	8	75		
Nauplius	108		96	50	60		96	25
Cyclops			24	25	12		8	
Diaptomus			8		4		4	
Daphnia	12	20	8	25			4	
Bosmina								

However, the very abundant Aphanizomenon is frequently well centrifuged. Table I shows twice as much in centrifuged surface and 4 meter samples as in trap-net samples, but an inconsistency at 8 meters and again a relatively very high count at 12 meters. Table II shows the same situation, but with a peculiar total absence of centrifuged spec-

imens at 12 meters. One might on the whole conclude that centrifuging was definitely the better method and that the net lost many. But Table III shows no such discrepancy between the two concentration methods.

TABLE III

CYANOPHYCEAE AND OTHERS

Numbers in 1 Liter of Turkeyfoot Lake Water, June 18, 1938

ORGANISMS	SURFACE WATER		4 METERS		8 METERS		12 METERS	
	Net	Centr.	Net	Centr.	Net	Centr.	Net	Centr.
Coelosphaerium	400		1,000		400		150	
Aphanocapsa								
Microcystis								
Oscillatoria								
Anabaena	10,600	1,000	9,800	1,000	9,800		800	
Aphanizomenon	9,600	6,000	8,600	8,000	5,400		200	
Melosira								
Synedra								
Asterionella			200		600	21,000	7,200	30,000
Fragilaria	600		200		200			
Stephanodiscus								
Staurastrum								
Pediastrum	800	60	48		28	80	32	
Ceratium	24,800	26,000	16,400	20,000	22,240	19,000	3,744	2,000
Polyarthra	156	20	156	60	296	100	124	
Keratella	36	40	20		48	40	36	
Synchaeta								
Asplanchna	16		24		12			
Notholca								
Nauplius			52	80	52	100	36	20
Cyclops			8	20	16		4	20
Diaptomus			24	20	48	60	8	
Daphnia	4		16		52		4	
Bosmina					4			

Statistically considered, the discrepancies of various sizes can be evaluated. Following methods elucidated by Ricker (1937, p. 74) and furthermore applied by him to certain of my examples in personal letter, a simple illustration can be given of Anabaena and Aphanizomenon in Table III.

The members per liter for the four depths can be added. This tabulation follows:

	Per Liter	Actual Count	Limits of Confidence	Confidence Limits per Liter of Lake Water
Net	30,100	155	132 to 181	26,400 to 36,200
Centr.	2,000	2	0.2 to 7.2	200 to 7,200

In the possible statistical ranges there is no overlapping whatsoever for the two methods. In other words, these discrepancies are proven significant, and it is positive that the centrifuged samples are of no value in the case of Anabaena. In the case of Coelosphaerium the same thing would be demonstrated. In corroboration was the evidence described previously of the losses in actual centrifuging.

But in Aphanizomenon, the picture is different.

	Per Liter	Actual Count	Limits of Confidence	Confidence Limits per Liter of Lake Water
Net	23,800	119	99 to 142	19,800 to 28,400
Centr.	14,000	14	7.7 to 23.5	7,700 to 23,500

These differences are not actually significant, because of overlapping of the statistical ranges secured from the two concentration methods.

To be sure, if Aphanizomenon counts of Tables I and II were taken as examples and similarly worked out, we would find in the statistical ranges of limits of confidence per liter of lake water of the two techniques, a real significant difference, but the centrifuge is higher.

Conclusions regarding Aphanizomenon are consequently difficult to make. Sometimes some seem to be lost in centrifuging, though generally not, and some may pass through the net, depending possibly upon massing of the filaments, if more isolated, passing through more freely.

The other plankton organisms of Tables II and III can be quickly compared. These and a score of others of the same station show innumerable small differences in counts calculated to numbers in one liter when net and centrifuge results are compared. The many differences found were disconcerting during this study, but statistical analysis will show most of them of little significance.

Diatoms when very abundant as in the normal spring maximum offer another problem. Tables IV and V demonstrate that while the net collection numbers are large and seem to show successful collection, with huge numbers and normal rises and declines in various parts of the year, the centrifuge numbers are, though practically only at the height of the season, still far greater than the net totals. At the maximum, a significant difference is proven, as in Asterionella, 1937, (Table IV) and Synedra, which, however, never attains the numbers of Asterionella at its maximum. In 1938 (Table V) Synedra reveals the same picture and even greater numbers, but Asterionella is relatively low

compared with a year before, and oddly the discrepancy between net and centrifuge counts is not so pronounced. But one month later, May, 1938, Asterionella reached a maximum though not duplicating that of April, 1937, but with the same discrepancies between net and centrifuge. Possibly when the numbers become enormous, more are pushed through the net meshes.

TABLE IV

DIATOMS

Numbers in 1 Liter of Turkeyfoot Lake Water, April 24, 1937

ORGANISMS	SURFACE WATER		4 METERS		8 METERS		12 METERS	
	Net	Centr.	Net	Centr.	Net	Centr.	Net	Centr.
Melosira	150			1,000	600			1,000
Synedra	7,650	21,000	4,400	27,000	3,000	18,000	2,600	31,000
Asterionella	140,100	856,000	136,200	1,139,000	126,000	955,000	94,400	811,000
Fragilaria	1,050	3,000	600	5,000	1,000	6,000	1,400	4,000
Stephanodiscus	600	2,500	300					3,000

TABLE V

DIATOMS

Numbers in 1 Liter of Turkeyfoot Lake Water, April 16, 1938

ORGANISMS	SURFACE WATER		4 METERS		8 METERS		12 METERS	
	Net	Centr.	Net	Centr.	Net	Centr.	Net	Centr.
Melosira	1,000		1,200		1,200	1,000	2,000	1,000
Synedra	45,000	64,000	50,200	76,000	55,000	224,000	40,600	178,000
Asterionella	18,200	12,000	26,000	13,000	15,400	19,000	19,000	3,000
Fragilaria	200	1,000	800	1,000	24		200	1,000
Stephanodiscus								

Entomostraca, being relatively gigantic plankton and also rotifers, are not present in such great absolute numbers, but do show normally large populations. There are no very significant differences between net and centrifuged records, though in some cases Entomostraca are missing in counts of centrifuge sample when present in small numbers in net samples, showing that the 1 liter water bottle sample is rather too small.

On the other hand, sometimes the centrifuge number, a round number, appears larger. This does not indicate that the centrifuge collection is better. Indeed it is less accurate than the net collection because based on fewer actual counted specimens and involving more

multiplication in the calculation. For example, (Table III) Nauplius at 8 meters has 82 in net sample and 100 in centrifuge sample. Actually, 13 were counted in the one c. c. from the 40 c. c. net sample; multiplied by four, showed 82 in a liter. But only 5 were actually counted in the one c. c. of the 20 c. c. centrifuge sample; multiplied by 20, would show 100 in a liter. The 100 is less accurate and probably a little too large.

Similarly in counts of smaller organisms when counted in 20 squares, when still more multiplication is necessary. For example, Fragilaria in Table II, at surface shows 800 in net and 3000 in centrifuge samples. Naturally, there is no proof in this case that some might not have gone through the net. In net sample 4 were counted in 20 fields; $4 \times 50 \times 4$ yields 800, the liter number. In the centrifuge sample 3 were counted in 20 fields; $3 \times 50 \times 20$ yields 3000, the liter number. In various scattered cases occur instances of appearance of 1000 supposed individuals per liter in centrifuge samples, in each case based on only one actually counted in the 20 squares of the cell. Invariably in those cases, the number 1000 is larger than the calculated number in net sample. Mostly the differences are small and not statistically significant. Nevertheless, it is more than a coincidence that such centrifuge numbers are larger than the respective net numbers. They are somewhat less accurate.

In plankton work, samples at least several times larger than one liter are advantageous, because in the small residual samples counted, there will be represented a larger liter fraction that is actually counted. Naturally the counting cell sample must not be too concentrated for accurate counting. Between any two samples secured by 2 collecting techniques, that sample is better which has a larger volume of its water surveyed and more of its plankters counted, and which requires the least multiplying to get the number per liter.

This is important, but it is not an indictment of the water bottle as such, but of the relatively small size bottle ordinarily used, as compared with the trap.

SUMMARY AND CONCLUSIONS

Plankton collections made with trap and with water bottle have ordinarily been handled as two separate entities with mutually exclusive groups of organisms counted from each.

In this study all plankton (except some obvious nannoplankton) was studied and compared throughout from trap and water bottle samples.

The comparison served as a check of the two methods, theoretically valid throughout the range of net-plankton.

Some organisms are difficult to classify into net plankton or nannoplankton.

The net (as is well known) allows real nannoplankton to pass through its meshes.

The water bottle (as is well known) retains all nannoplankton.

Many small differences occur between samples concentrated by the net and by the centrifuge, though most of these are not statistically significant.

Water bottle samples as made in this study and frequently otherwise, are too small in quantity compared with the 10 liter trap samples. If the bottle samples were larger there would be more and more offset to the disadvantage next listed.

The larger the portion of any collected sample which has its plankton actually counted and the less multiplication required to secure numbers per liter, the better the results. In that sense the trap-net samples are better than the centrifuge samples when done in the manner described.

The centrifuging process at 15,000 r. p. m. introduces some problems, in breaking up some organisms, thus adding to inaccuracy of counts, and moreover in failing to retain in the centrifuged sample, many important blue-green algae of low specific gravity, especially of the summer collections.

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