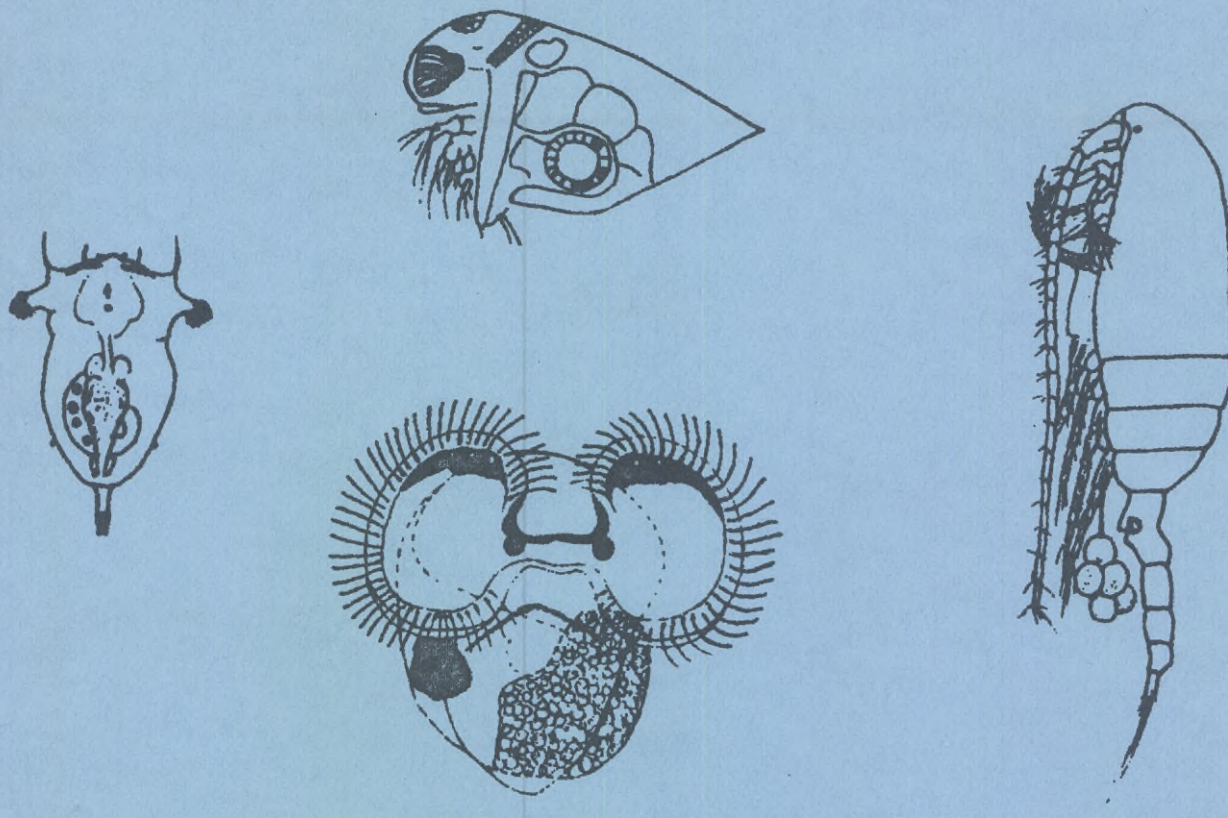

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Zooplankton biomass estimation - a short description and comparison of two methods

by

Lars Hernroth

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Institute of Marine Research,
S-453 00 Lysekil, Sweden.

INTRODUCTION

In planktonic research, as in many other fields, the lack of uniform methods is quite annoying. The reasons for having so few generally accepted gears and analysing methods are very often due to the fact that a certain laboratory or country by tradition has used a certain method. A change of gear or method would therefore lead to new results, difficult to compare with the old ones. Another reason for the different methods might be as simple as the cost involved in changing to new equipment. The use of different gears and methods will of course lead to difficulties when comparisons have to be made between the results obtained by different scientists. These problems originate from the fact that very few intercalibrations and comparative tests between the different methods have been done.

The increasing co-operation between the marine scientists in the countries surrounding the Baltic have showed the necessity for such intercalibrations and comparative tests. The scientific issues are namely often the same but the translation of the results into a common language have often proved to be difficult.

One of the issues that has been of great interest to the Baltic zooplanktologists is that of estimating the zooplankton biomass. Through the years a variety of methods have been applied ranging from the rough and easy-to-handle methods like the settling volume technique to detailed and laborious methods like the chemical and biochemical. In general, five methods of estimating the zooplankton biomass have been used as standard procedures (Beers 1972). These methods are: (1) volumetric methods like settling volume and displacement volume, (2) gravimetric methods like estimates of dry weight, ash-free dry weight and wet weight, (3) chemical and biochemical methods which measure the content of specific elements, (4) calorific methods which measure the energy content and (5) biomass estimates which are based on size measurements of single specimens. The method used by the author have mainly been the time-consuming technique of counting each specimen and calculating the sum of all individual volumes. Due to the thoroughness of these analyses the time needed for each sample was extremely long. It was therefore logical to look for another method that faster, but with an acceptable accuracy, could measure the biomass. The method chosen was that of displacement volume mainly because this method is widely used by other Baltic zooplanktologists and also because it is recommended by the Baltic Marine Biologists (BMB publ. no. 1: Recommendations for Marine Biological Work in the Baltic.-In press.).

This paper deals with a description of the two methods as well as a comparison of the results obtained.

MATERIAL AND METHODS

Since 1968, the Institute of Marine Research in Lysekil has regularly collected zooplankton samples at 7 off-shore stations in the Baltic proper (fig. 1). The stations are supposed to represent seven sub-areas of the Baltic and each station has been visited 4 times per year.

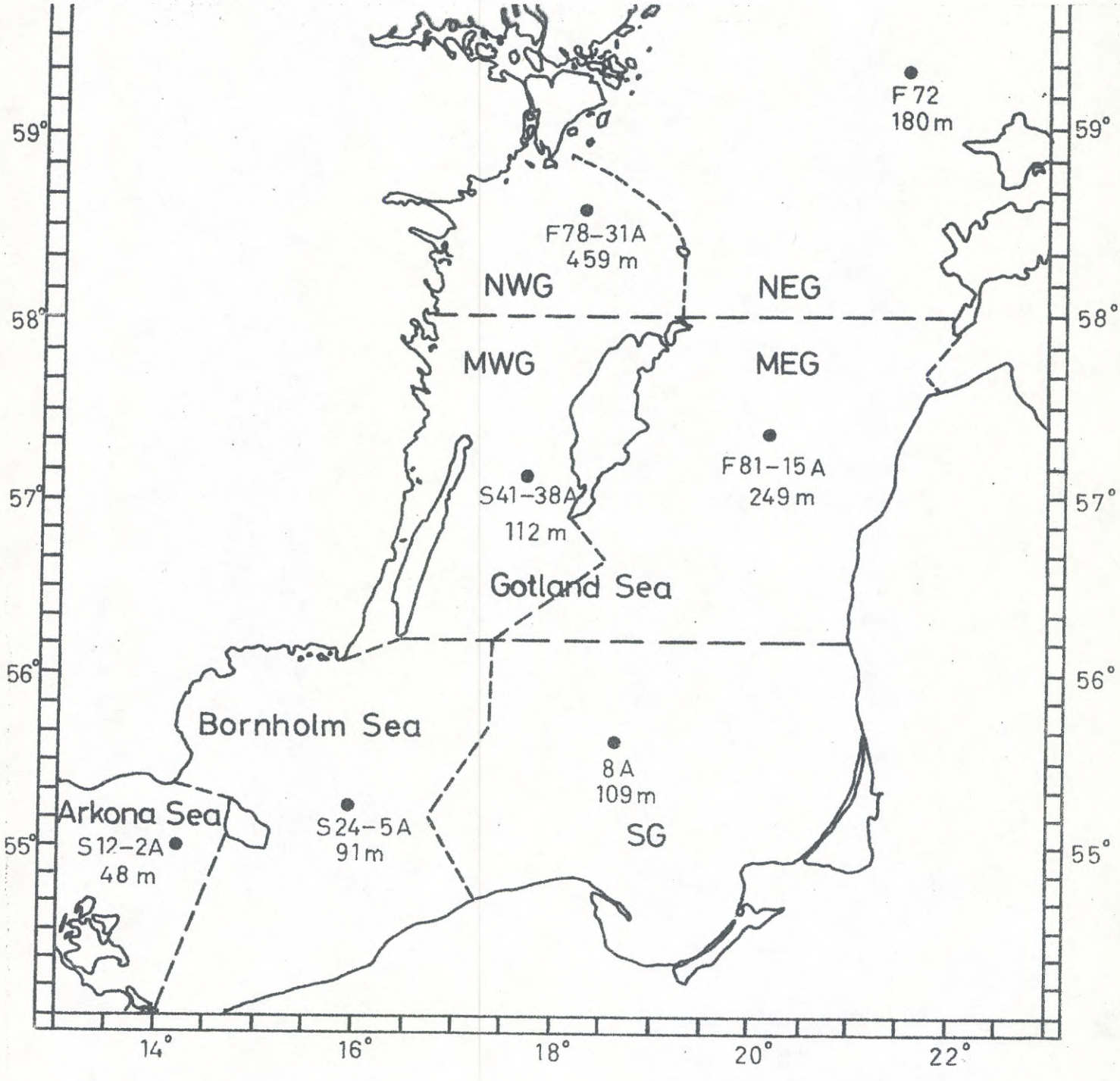

Fig. 1. Chart illustrating the 7 stations used by the Institute of Marine Research in Lysekil in zooplankton investigations.

From 1968 to 1973 the samples have been collected by vertical, fractionated hauls with a Nansen net of 160 µm and from 1974 and on an Unesco WP-II net of 200 µm has been used. All samples have been preserved in 4% formalin. From each sample, two subsamples (2/100th or 2/10th) were taken by use of the whirling apparatus constructed by Kott(1953). In the sub-samples, all specimens were determined to species and the copepods also to developmental stage.

The biomass was calculated by the technique used by Lohmann (1908) specifying the volume of each species and developmental stage and assuming a density for zooplankton of 1 g/cm^3. Biomass can then be expressed as gram wet weight. The specification of individual volumes that has been used is one that has been worked out especially for the Baltic by the Water Conservation Laboratory of Helsinki with a few additional values calculated by dr. H. Ackefors (table 1).

When using the displacement volume technique for biomass estimation the preserved sample is diluted to a specified volume (50 or 250 ml) which is poured through a sieve of the same mesh-size as the plankton net. The sample is then retained by the sieve and the remaining volume of water is collected in a measuring glass. The interstitial water is drained from the sample by use of a vacuum-pump (fig.2). The difference between the two volumes is then equal to the volume displaced by the sample. It must be pointed out however, that this volume does not always contain purely zooplankton but often also phytoplankton and to some extent interstitial water.

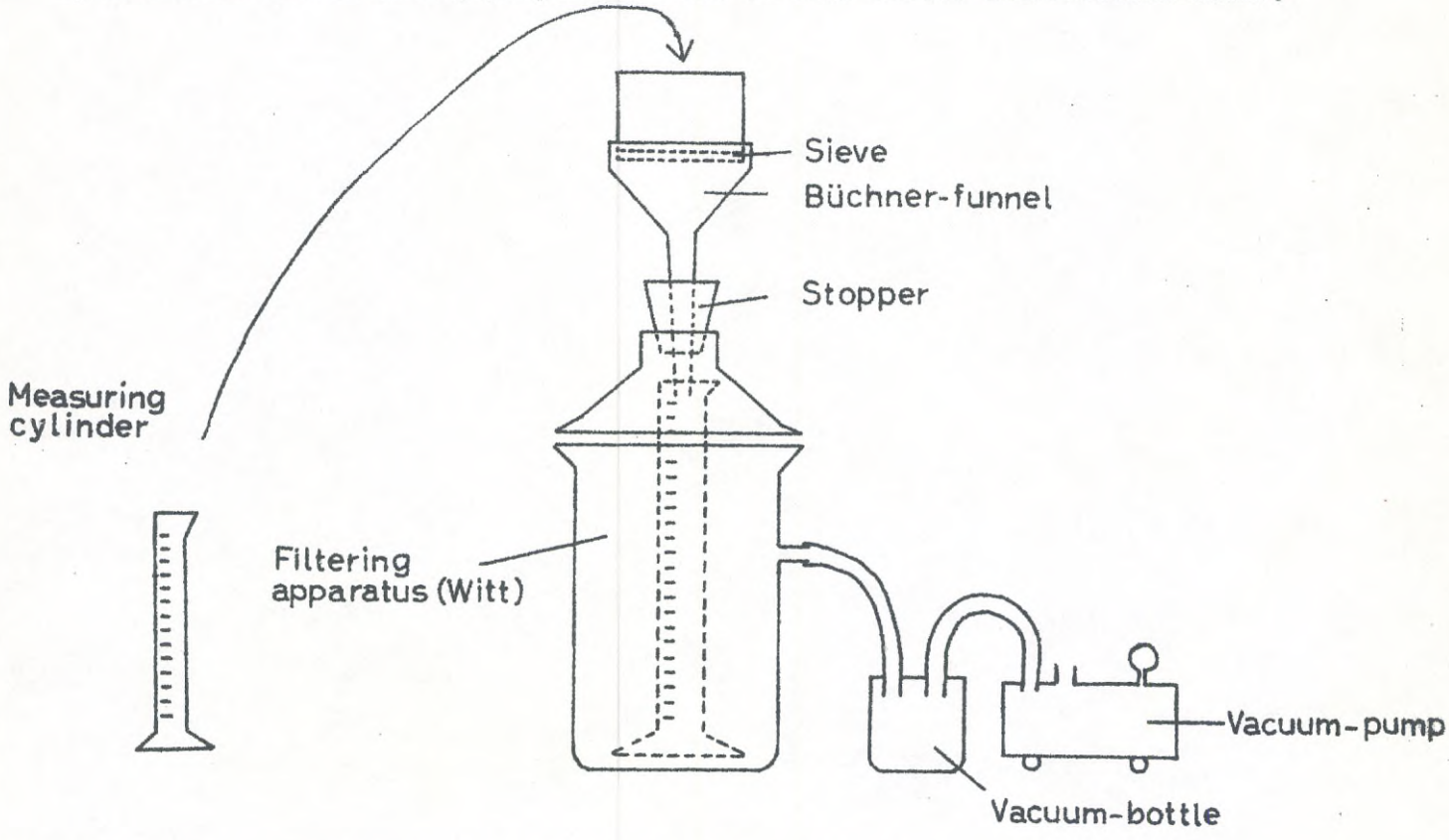

Fig. 2. Apparatus used in displacement volume technique.

Table 1. Calculated volume of zooplankton in μ^3. The values are estimated by The Water Conservation Laboratory of Helsinki. All values marked with figure 1 are estimated by H. Ackefors.

			Volume in μ^3
Aurelia aurita	Ephyra larva	ø 5 mm	10 000 000 000[1]
		ø 6 mm	14 000 000 000[1]
Cyanea capillata	Ephyra larva	ø 7 mm	20 000 000 000[1]
		ø10 mm	40 000 000 000[1]
Pleurobrachia pileus	Cydippid larva	ø 0.7 mm	180 000 000[1]
Keratella quadrata quadrata			200 000
" " platei			200 000
" cochlearis recurvispina			76 000
" cruciformis eichwaldi			76 000
Synchaeta spp.			2 000 000
Harmothoe sarsi			12 000 000
Bosmina coregoni maritima			10 000 000
Podon intermedius			20 000 000
Podon polyphemoides			10 000 000
Podon leuckarti			10 000 000
Evadne nordmanni			10 000 000
Limnocalanus macrurus (L.grimaldii)		ad.	400 000 000
" "		cop.stage	50 000 000
" "		naup.stage	1 000 000
Acartia bifilosa & A. longiremis		ad.	80 000 000
" " "		cop.stage	10 000 000
" " "		naup.stage	1 000 000
Eurytemora sp.		ad.	77 000 000
"		cop.stage	10 000 000
"		naup.stage	1 000 000
Centropages hamatus		ad.	80 000 000
" "		cop.stage	10 000 000
" "		naup.stage	1 000 000[1]
Pseudocalanus m. elongatus		ad.	160 000 000[1]
" "		cop.stage	20 000 000[1]
" "		naup.stage	2 000 000[1]
Temora longicornis		ad.	80 000 000
" "		cop.stage	10 000 000
" "		naup.stage	1 000 000
Cyclops spp.		ad.	30 000 000
"		cop.stage	8 000 000
"		naup.stage	470 000
Oithona similis	average value for all stages		3 000 000
Harpacticoida		ad.	8 000 000
"		cop.stage	2 000 000
"		naup.stage	500 000
Balanus improvisus		naup.stage	10 000 000
" "		cypris stage	52 000 000[1]
Mysis relicta		size 15 mm	90 000 000 000[1]
Hyperia galba		size 6 mm	16 000 000 000[1]
Gastropoda		larva	1 000 000[1]
Lamellibranchiata		larva	1 000 000[1]
Sagitta elegans baltica		length: 20 mm	45 000 000 000[1]
Fritillaria borealis			10 000 000[1]

During the years 1971 and 1972 both of the described biomass methods were used on the samples collected. It has therefore been possible to make a direct comparison between the two methods using a fairly large number of samples (170). The relationship between the two methods was studied by means of regression analysis. The samples were divided into 8 groups representing 4 seasons and two years. For each group the displacement volume was regressed on the sum of individual volumes. The calculated regression lines were tested for homogeneity of slope (Sokal & Rohlf 1969). As no inhomogeneity was shown between the years within season, the two years were averaged. The resulting four lines are described in table 2 and fig. 3.

RESULTS

When comparing the two biomass methods, rather uniform results were obtained during the seasons of low productivity (winter, spring and autumn). This is illustrated in fig. 3. No difference on the 95% level of confidence was found between the three seasons. As a consequence, winter, spring and autumn could be treated jointly, separated from summer. The equation for each line of regression ($y=a + bx$, where y= displacement volume, a= point of interception, b= coefficient of inclination and x= individual volume) as well as the mean for all three seasons is found in table 2. From this table it is evident that the relationship between the individual volume technique and the displacement volume technique is directly proportional using samples from winter, spring and autumn ($y=bx$, a is negligible). The mean coefficient of inclination for winter, spring and autumn (b_{wsa}), was found to be 1.30 which means that the displacement volume technique will give approx. 30% higher values during these seasons. Using samples collected during summer will on the other hand give a different equation where the relationship between the individual volume technique and the displacement volume technique is <u>not</u> directly proportional but dependent on both a and b ($y=a + bx$). In this case the use of small samples (1-10 g) will give a poor relationship due to the relatively high value on a. However, as the biomass of the samples increases, the agreement between the methods increases in a corresponding way. In the range 15-35 g, which is a typical summer range, the difference between the two methods is very small, $\pm$ 7%.

Table 2

	b	a	s_{yx}	
Winter :	1.54	-0.30	1.14	y=1.54x - 0.30
Spring :	1.29	-1.19	3.75	y=1.29x - 1.19
Summer :	0.82	+3.88	4.52	y=0.82x + 3.88
Autumn :	1.21	+0.93	1.64	y=1.21x + 0.93
Winter-spring-autumn:	1.30	-0.12	2.81	y=1.30x - 0.12

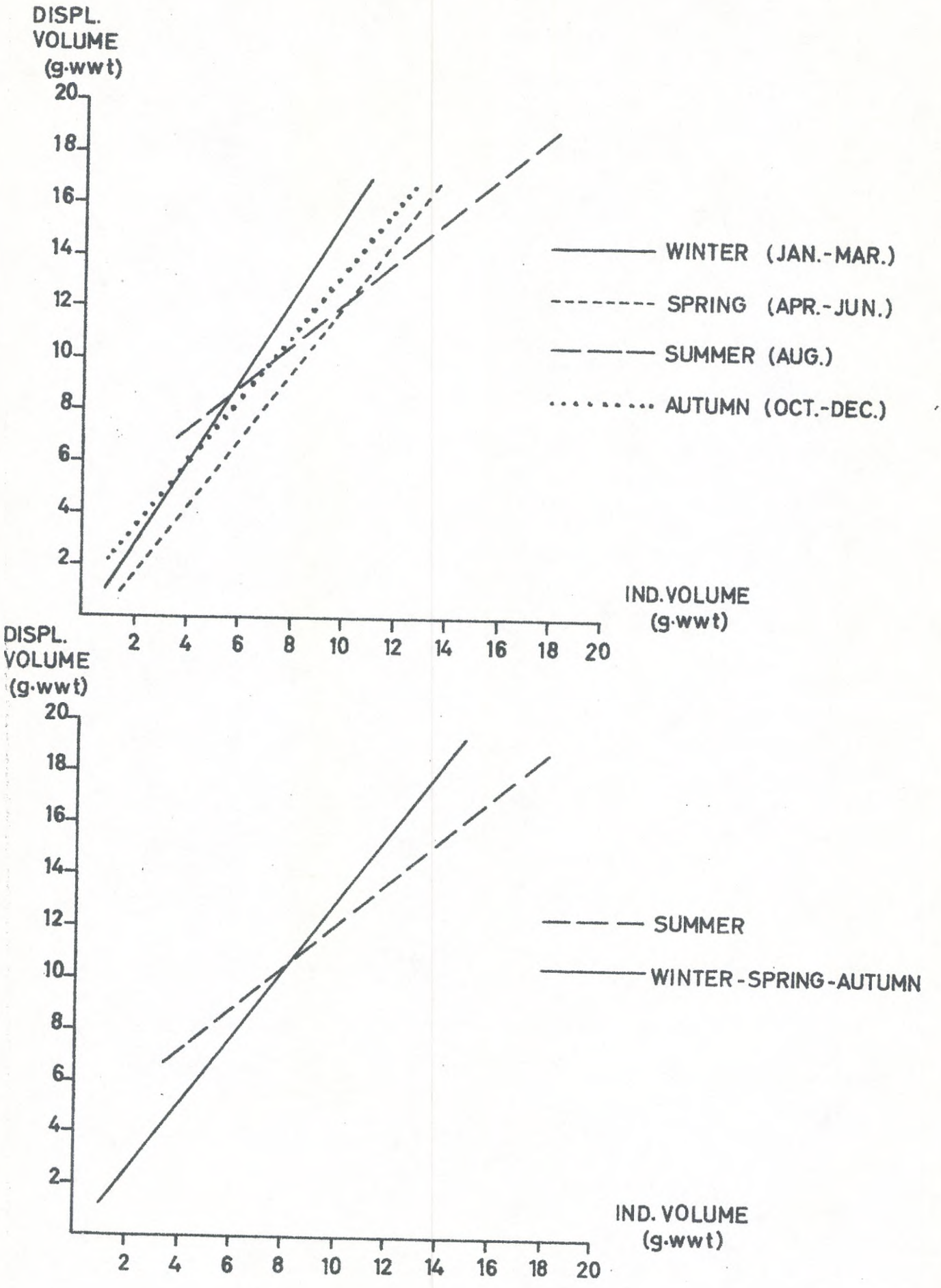

Fig. 3. Regression analysis of two methods to estimate zooplankton biomass.

DISCUSSION

One can predict, that a technique using displacement volume will always give a value higher than the true value. The interstitial water can hardly be drained to 100% and the influence of particles other than zooplankton can never be overlooked. (A value less than the true value can of course be obtained if the vacuum is so low that the organisms are damaged and drained of normal body fluids). The precision of the technique is therefore very much dependent on the apparatus used. The technique used in this investigation must be considered rough, mainly because of the measuring-cylinders used which are hard to read at a better accuracy than 0.5 ml and partly because of the varying amount of water stuck to the walls of the cylinders by surface tension. As a consequence of this, small volumes will be impaired by rather large errors. These errors will be of less importance as the biomass increases however.

To reduce these technical shortcommings a more accurate apparatus has been constructed by Lillelund & Kinzer (1966) which works in about the same way but with a better accuracy in the measuring part (fig. 4). The value of 30% difference that was found in the seasons of low productivity ought to change if this more accurate apparatus is used.

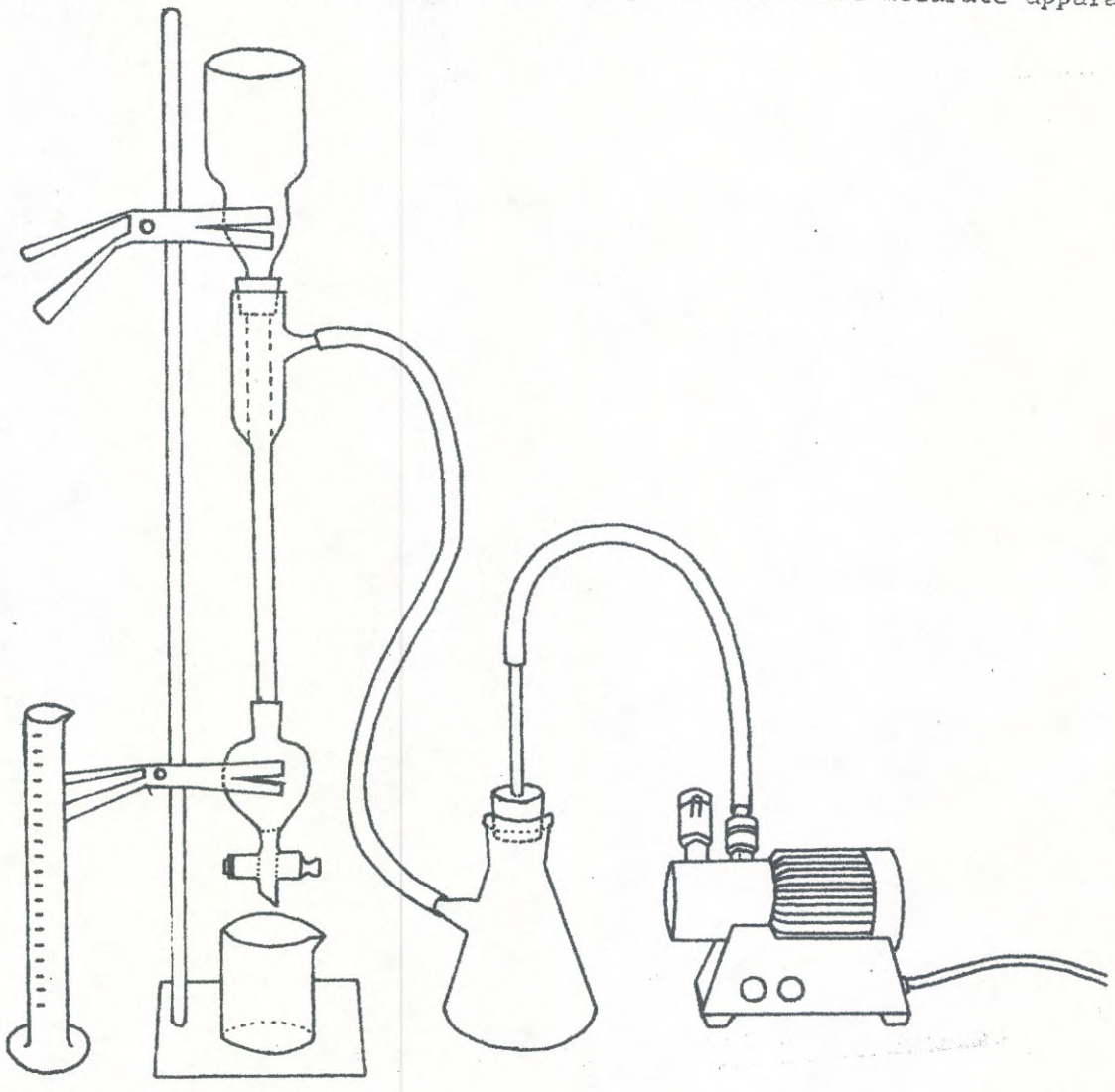

Fig. 4. Displacement volume apparatus constructed by Lillelund & Kinzer.

As was pointed out, the influence of these technical sources of error is reduced as the volume of the sample increases. This is proved by the results obtained from the summer samples where the biomass-range 15-35 g showed a very good agreement between the two methods ($\pm$ 7%). An attempt to explain the high value of a (3.88) when using summer samples would be the presence of blue-green algae which most summers influence zooplankton samples. The more or less constant amount of blue-green algae in each sample would thus cause the regression line to intercept at 3.88 instead of origo as would be the case in a "clean" zooplankton sample. Consequently, the presence of blue-green algae will cause a greater error in small samples (<10 g) than in larger ones.

The question whether the technique of calculating the sum of all individual volumes will give a "true" value or not is naturally dependent on the accuracy of the estimation of each individual volume. The advantage of this method is the thoroughness in the analysis which eliminates the error of both interstitial water and particles other than zooplankton but the disadvantages are obvious. The time needed for analysis and calculation is at least 10 times that of the displacement volume technique.

CONCLUSIONS

The technique of displacement volume will give a value 30% higher than that of individual volume technique when using samples from seasons of low biomass (< 10 g). As the biomass values increase, the agreement between the methods increases and the best results ($\pm$ 2%) have been found during summer with biomass values around 20 g. In view of the results obtained in this investigation, the use of a simple and easy-to-handle displacement method can in many cases be justified. The lower accuracy is well compensated by the capacity of the method which is about 10 times that of the individual volume technique.

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