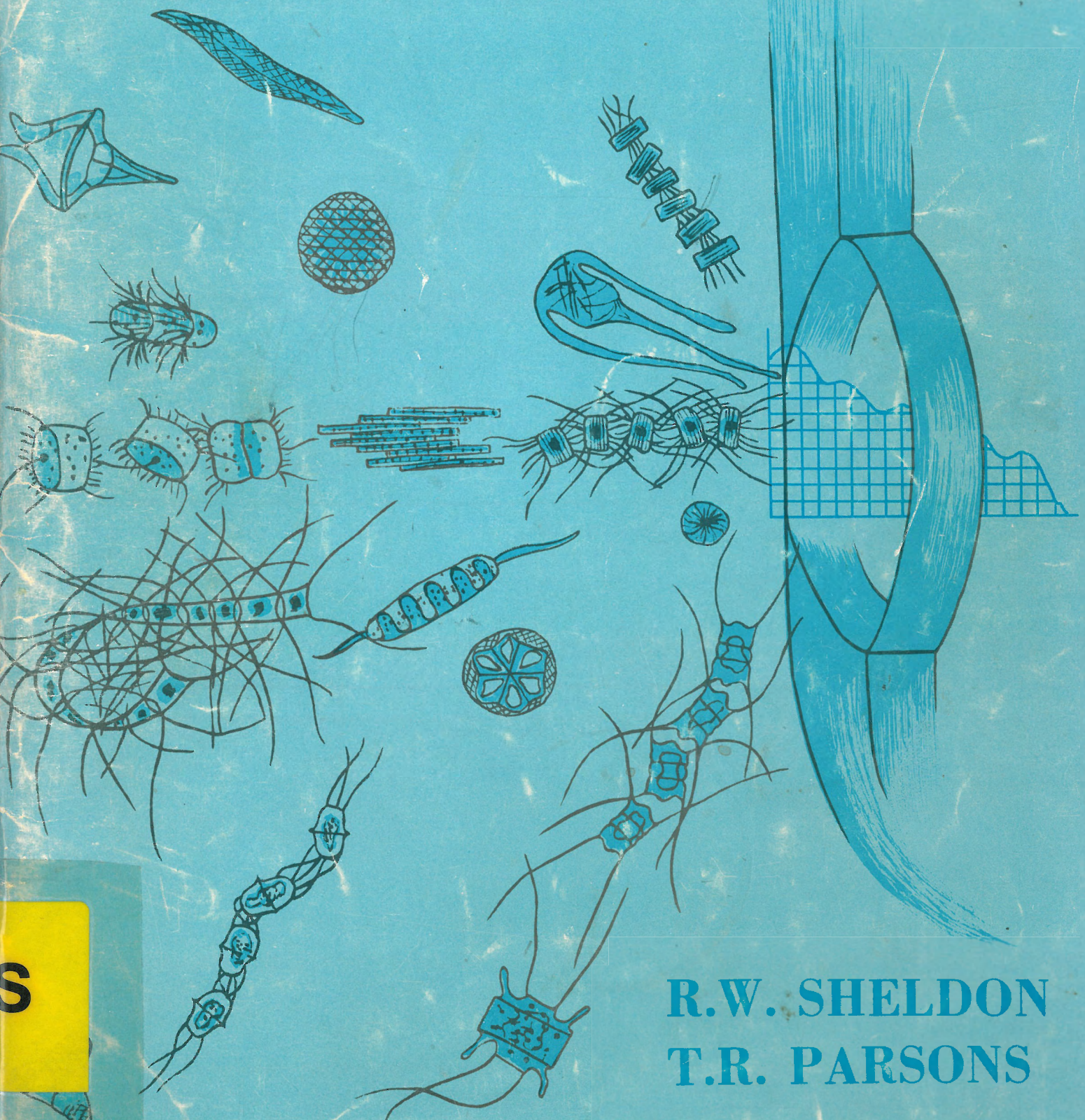
A PRACTICAL MANUAL ON THE USE OF THE COULTER COUNTER IN MARINE SCIENCE



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A PRACTICAL MANUAL ON THE USE OF THE COULTER COUNTER IN MARINE RESEARCH

by

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PREFACE

This manual is published by Coulter Electronics Sales Company-Canada as a service to marine science. Some of the methods are from a previous report by the authors (Manuscript Report No. 214, Fisheries Research Board of Canada, 1966) and this manual represents continuing refinements of techniques utilized in modern oceanographic research.

Most of the techniques were developed at the Pacific Oceanographic Group of Fisheries Research Board of Canada. Coulter Electronics Sales Company-Canada is pleased to recognize the contributions of Drs. Sheldon and Parsons, and of their employers, to the field of electronic counting and sizing of organic and inorganic particulate material.

I believe that this manual will be useful to marine scientists of many disciplines who study suspended and sedimented material in sea water. In many cases the same techniques may be applied to limnological studies.

Finally, although the methods described refer specifically to the Model B Coulter Counter®, many can be applied to the earlier Model A or Model F, and will also be applicable to any foreseeable development of the Coulter Counter®.

Toronto, Ontario
13 March 1967

Dale A. Christensen, General Manager
Coulter Electronics Sales Company-Canada

the Coulter Counter.

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SECTION A.

DESCRIPTIVE

AND

THEORETICAL

I. INTRODUCTION

The Coulter Counter was originally designed for routine counting of red-blood cells, but it has found many other applications, not only in medicine but also in the biological sciences and in industry. The amount of information available on uses other than in routine medical work is now considerable.

It is surprising, in view of the extensive applications elsewhere, that the Coulter Counter has not been more widely used in marine science. The reason for this is that in marine work many of the problems requiring particle counts are very different from those in other fields of research. Particle distributions commonly found have two basic characteristics; the particles are often at low concentrations and nearly always cover an enormous range of size. It is because of this that many of the techniques developed in biology, medicine, and industry cannot be applied directly to marine studies. There are certain tasks to which the Coulter Counter has been applied with success (e.g., counting unicellular algal cultures), no doubt because the methods do not differ greatly from those for blood-cell counts. But in general, if techniques developed in other fields are applied directly to marine problems the results are often discouraging. However, with only small changes in counting procedures and a few additions and adaptations of apparatus, a model B Coulter Counter can be made to give results which, at present, cannot be obtained in any other way.

It is the purpose of this manual to describe the practical operation of the model B Coulter Counter and to show how it can be used to advantage in marine research. However, descriptions of techniques, no matter how detailed, can never be a substitute for experience. We would strongly advise that an operator spend some time gaining experience with the instrument before making observations of any importance.

II. DESCRIPTION OF THE INSTRUMENT

Several good descriptions have been published (e.g., El-Sayed and Lee 1963; Maloney et al 1962), and although these are of an older and less useful instrument - the model A - the principle and operation also apply (with minor differences) to the model B. A full description both of the principle and operation of the model B is given in the instruction manual supplied with the instrument. The description given here will be brief, and it is included so that the rest of the manual can be readily understood.

10 / *Description of The Instrument*

The basic principle on which the instrument operates is very simple. If an electric field is maintained in an electrolyte, then a particle passing into this field will cause a change in electrical properties if the resistivity of the particle differs from that of the electrolyte. The particle will displace its own volume of electrolyte and the change produced will be proportional to particle volume. The relationship between change in electrical properties and particle volume is linear.

The arrangement of the instrument is shown diagrammatically in Fig. 1. To operate, the tap on the glassware is opened and mercury is drawn into a manometer by means of a small pump. The tap is then closed and the mercury falls back. As it does so, it takes with it a suspension of particles. This has to pass through a small aperture in a glass tube, across which an electric field is maintained. As particles pass through the aperture they cause electrical disturbances. These are measured and counted automatically.

The purpose of the aperture is to limit the volume of electrolyte in which changes of electrical properties can be produced. It has been found in practice (Mattern et al 1957) that the electrically sensitive zone is about three times the volume of the aperture and comprises the aperture itself and two hemispheres of similar volume (Fig. 1).

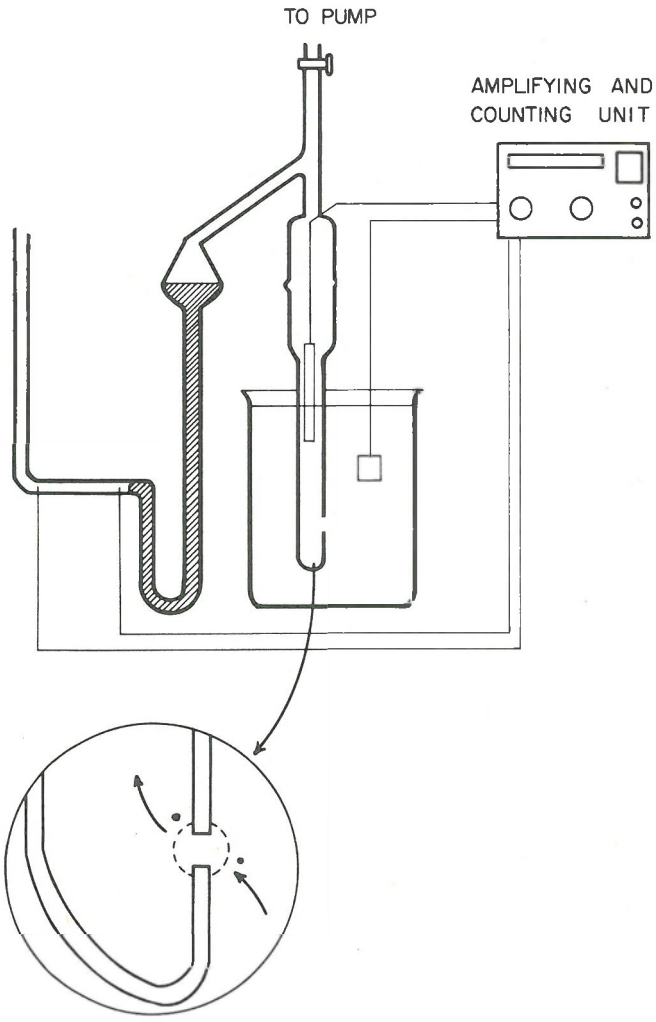


Fig. 1

As the mercury falls through the manometer it operates two switches fused into the glass. The first starts the counter and the second stops it, so that particles in a definite volume of sample are counted.

The sensitivity of the instrument can be varied either by changing the degree of amplification of the electrical disturbances (or pulses), or by changing the strength of the current between the electrodes. The amplification can be varied step-wise by factors of 2 and the aperture current by factors of 2 or $2^{\frac{1}{2}}$. Relatively small particles can be counted at high sensitivity and relatively large particles at low sensitivity.* The range of particle size over which counts are made can be limited by means of threshold controls. These are electrical filters. The upper threshold control permits only pulses (i.e., particles) less than some pre-determined value to be counted, and the lower threshold control will allow only pulses greater than some other pre-determined value to be counted. Counts can therefore be made over a definite range of particle size.

As oscilloscope is provided so that a rough visual check can be made of the particle size distribution. Electrical pulses are seen as vertical lines, the length of which is proportional to particle size. The effect of changes in sensitivity and threshold settings can easily be seen.

The presence of an aperture places certain restrictions on the size of particles which can be measured. The linear relationship between electrical pulse strength and particle volume does not apply if particle diameters are greater than about 40% of the aperture diameter. At the other end of the scale, pulses from particles with diameters less than 2% of the aperture diameter cannot be separated from electrical "noise". without "blank" circle.

Theoretically it is possible – by a suitable choice of aperture size, sensitivity, and threshold settings – to count particles between virtually any limits of particle size. In practice, inevitable restrictions of instrument design and experimental procedures limit the range which can be measured to particle diameters from about 0.5 μ to 1000 μ.

orifice	range (radii)	range (vol.)	range (x-sect)
350μ	3.5μ - 70μ	180 - 1436758μ^3	38.5 - 15394μ^2
100μ	1μ - 20μ	4.19 - 33510μ^3	3.14 - 1257μ^2
50	0.5μ - 10μ	0.52 - 4189μ^3	0.785 - 314μ^2

*It is convenient to consider instrument sensitivity as the product of Aperture Current and Amplification settings. For most practical purposes the settings are interchangeable, i.e., Amp. ½ Ap. Cur. 2 (Sensitivity = 1) is the same as Amp. 4 Ap. Cur. ¼ (Sensitivity = 1). Note that as the Amplification and Aperture current settings are reciprocals, small numbers signify high sensitivity and, therefore, small particles.

III. CALIBRATION

(1) GENERAL STATEMENT

It is necessary, before particle size can be measured, to calibrate the instrument. Calibration does not involve any of the electronic components of the instrument. Its purpose is simply to define the relationship between particle volume and electrical pulse strength for each aperture. Ideally a calibration should be worked out for each kind of particle, but in practice this is unnecessary. If we consider only one particle size and one aperture the general expression for resistance change in the aperture (see "Theory of the Coulter Counter") can be simplified to: $\Delta R = K(1 - \frac{\rho_\epsilon}{\rho})$ where ΔR is the resistance change caused by the particle, K is a constant, ρ_ϵ is the electrolyte resistivity, and ρ is the particle resistivity. If the particle resistivity is much greater than the electrolyte resistivity the term $\frac{\rho_\epsilon}{\rho}$ can be neglected and the change in resistance is then independent of particle resistivity. Most particulate materials met with in marine research have resistivities thousands of times greater than that of seawater – the commonly used electrolyte. This means that for practical purposes a single calibration will suffice for all particles.

(2) PROCEDURE

The actual procedure for calibration is very simple. It is possible to relate the calibration of one aperture to that of any other and ideally only one calibration need be made. However, as the process of calibration is not very time consuming it is preferable to calibrate each aperture separately. Calibration can be carried out very accurately with any kind of particulate material provided that particle diameters are within the range from 2% to 40% of the aperture diameter and the concentration (by volume) of the particulate material is known, (see "Theory of the Coulter Counter"). However, it is easier, and sufficiently accurate for most studies to calibrate using mono-sized particles. Pollen grains are commonly used. These are essentially mono-sized for each species; many species are nearly spherical, and they are available over a wide range of size. A list of some suitable calibration materials is given below.

Material	Approximate Size	Apertures to be calibrated
Plastic Spheres (Dow Chemical Co.)	3.49 μ	30 μ and 50 μ
Paper mulberry pollen	16 μ	70 μ and 100 μ
Ragweed pollen	19.5 μ	100 μ to 280 μ
Pecan pollen	45 μ	280 μ to 560 μ
Corn pollen	90 μ	400 μ to 1000 μ
Crab eggs	400 μ	2000 μ

The procedure for calibration using sea water as the electrolyte is as follows:

1. Select a pollen or other particulate material, preferably with the mean particle diameter between 10% and 20% of the aperture diameter, and disperse in particle-free sea water (see C.I(1) and C.I(2)). Leave for 24 hours.

2. Measure with a microscope the diameter of several hundred pollen grains, and calculate the mean diameter and volume.

3. Fill the aperture tube with sea water.

4. Measure the resistance across the aperture. This measurement is not critical and can be made with an ordinary multi-purpose meter. Put the aperture current switch to OFF, put one probe on the external electrode and one on the metal shield around the sample platform and record the resistance.

5. Set the matching switch – this is located inside the cabinet above the oscilloscope – according to the following table.

Aperture resistance (ohms)	Matching switch setting	Minimum aperture current switch setting
10,000	128–H	1/8
	128–L	1/8
20,000	64–H	1/4
	64–L	1/4
40,000	32–H	1/2
	32–L	0.354
80,000	16–H	1
	16–L	1/2
160,000	8–H	2
	8–L	1
320,000	4–H	4
	4–L	2

The matching switch should normally be set on the H position. With electrolytes giving high aperture resistance it might be necessary to experiment to see whether the instrument works best with the switch in the H or the L position. Aperture resistances with seawater are usually less than about 20,000 ohms (50 μ aperture 20,000 ohms, 560 μ aperture 5,000 ohms).

6. Record the minimum permissible aperture current switch setting. It is important not to use settings less than this because the relationship between electrical pulses and particle volume will not be linear.

(Older counters may not have a matching switch, for these ignore 4, 5, and 6).

7. Check that upper threshold mode switch is in SEPARATE position.

8. Make up a suspension of pollen grains (see C.I(1)) at low concentration with particle-free sea water (see C.I(2)). The maximum recommended concentrations are given in Fig. 2. Concentration can be checked in the following way. Set the amplification and aperture current switches so that most of the pulses reach between 1/3 and 2/3 up the oscilloscope screen. Set the lower threshold at about half this value (i.e., 15–30) and switch off the upper threshold control. This is done simply by turning the control as far as it will go past the 100 mark. Counts taken at these settings will include most of the particles in suspension, and particles should not be at greater concentrations than shown in Fig. 2.

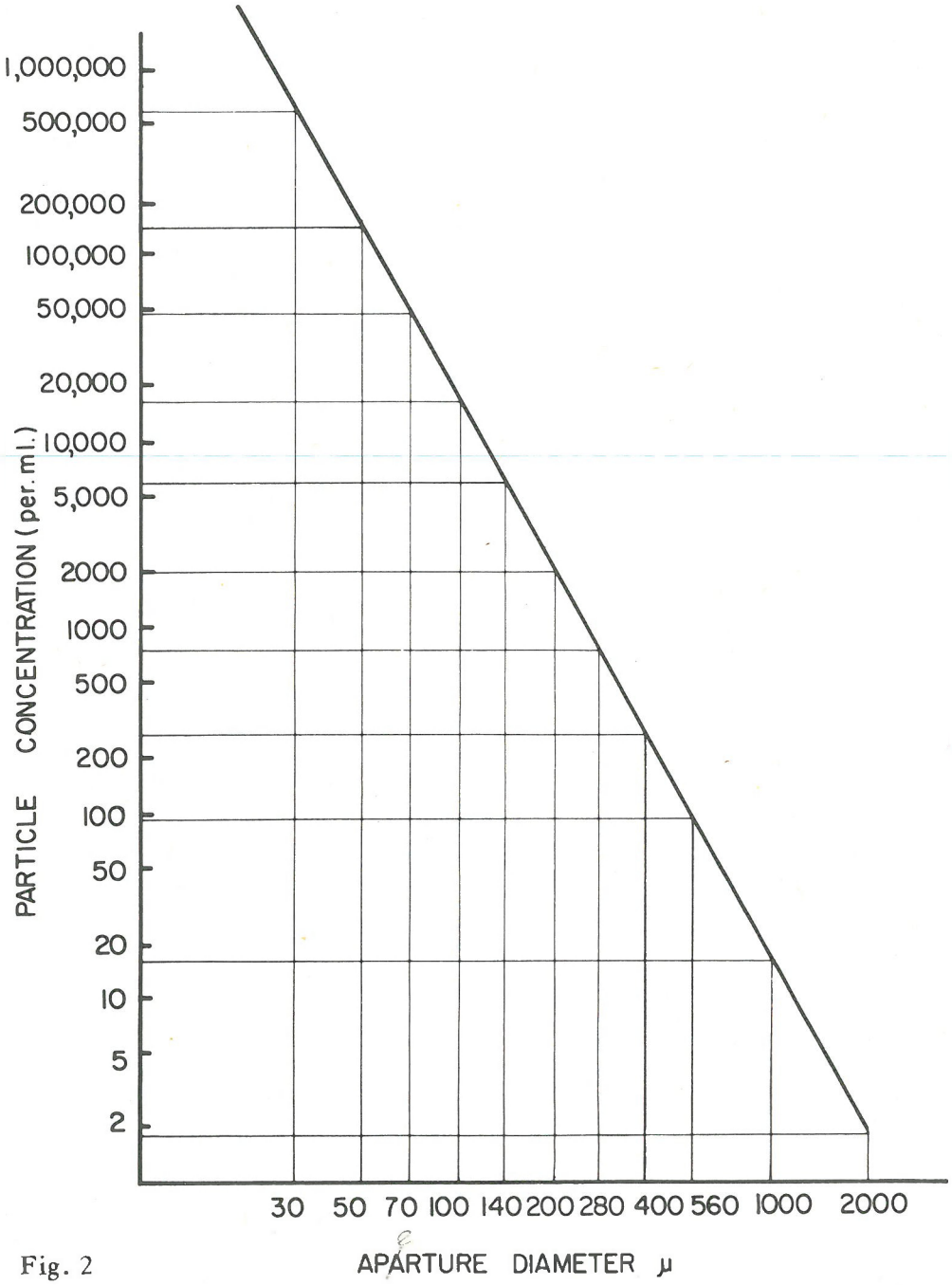


Fig. 2

9. Record the aperture current and amplification settings used in 7.

10. Leave the upper threshold switched out, and set the lower threshold at 10. Make counts at this setting, and then at convenient successively larger threshold settings. Each of these counts will be of all particles in suspension larger than the particle size (as yet unknown) represented by the lower threshold setting.

11. A plot of count against threshold setting will give a cumulative frequency distribution (Fig. 3), and the median is a measure of average particle volume. (For calibration of very small apertures see C.I(3)).

12. We have now established one point on the threshold scale where particle volume is known. As the relationship between threshold setting and particle volume is linear, the particle volumes represented by all points on the threshold scale are known. This is for the instrument at one particular sensitivity. However, as each Aperture Current and Amplification setting alters sensitivity by a factor of 2; then particle volumes represented by threshold settings at all sensitivities are known.

Example. In Fig. 3, we have seen that at sensitivity 1/4 (Aperture Current 1/2 and Amplification 1/2) 1 threshold division represents 111.4 μ^3. At sensitivity 1/8 (Amplification 1/4 Aperture Current 1/2) 1 division represents 55.7 μ^3, and at sensitivity 1/2 (Aperture Current 1 Amplification 1/2) 1 division represents 222.8 μ^3. This is summarized in Fig. 4.

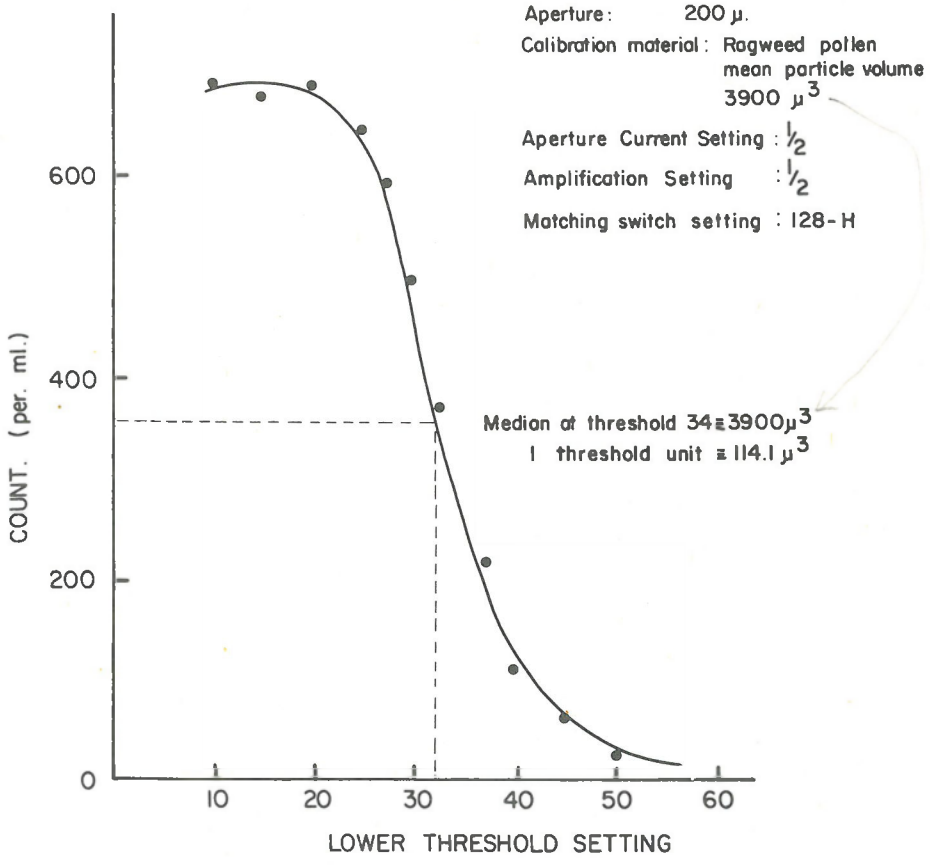


Fig. 3.

It is sometimes convenient for 1 threshold division to represent a pre-determined particle volume. For instance, in the example shown (Fig. 3) it might be convenient for calculation purposes for 1 division to represent 100 μ^3, instead of 111.4 μ^3 as found. There is a control inside the cabinet (variable gain control) which over the whole of its range will alter sensitivity by a factor of 2. It is, therefore, possible to select sensitivity settings between the 2 x steps of the aperture current and amplification controls. The effect of the variable gain control is illustrated in Fig. 4. The correct setting for this control is found by trial and error, and once set should not be changed. Moving this control changes the calibration, and it is difficult to set back exactly in the same place. Unless a definite threshold division to particle volume relationship is required the variable gain control should be left in the middle of its range.

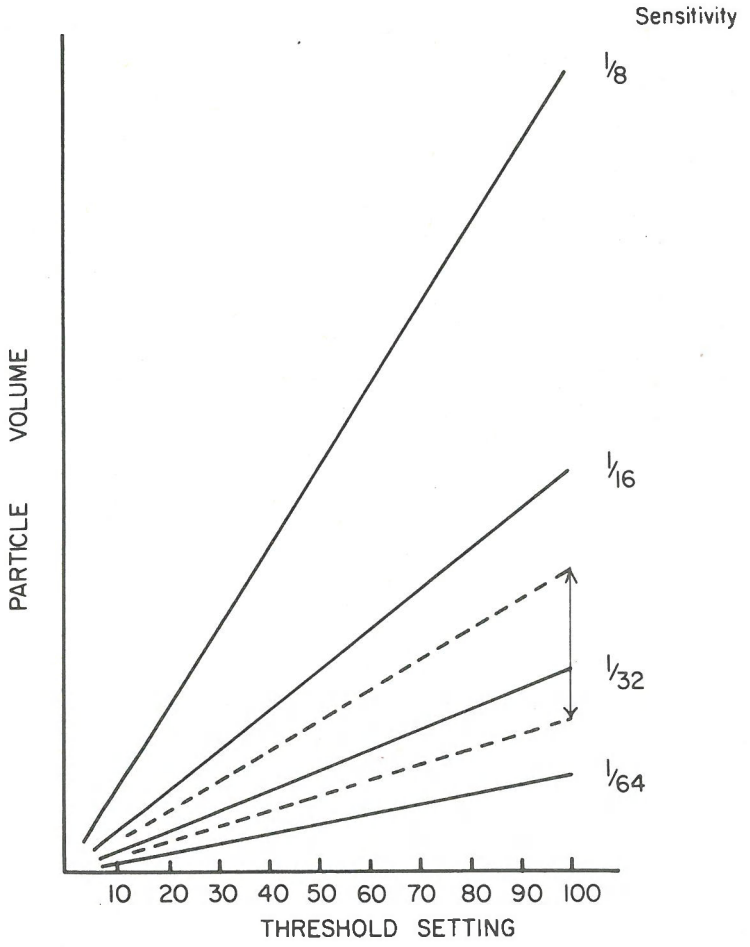


Fig. 4

Having now defined the exact relationships between instrument settings and particle volume there are 3 ways in which the calibration may be written out:

Arithmetic scale of particle volume: This is the easiest scale to use and is obtained directly from threshold settings. It should only be used for particle populations where the difference between smallest and largest particles is relatively small. (Ratio of about 1:20

Diameter (μ)	Log_{10}D	Volume (μ^3)	Volume (μ^3)	Log_{10}D	Diameter (μ)
1.00	0.00	0.52			
1.26	0.10	1.04	0.73	0.05	1.12
1.58	0.20	2.08	1.47	0.15	1.41
2.00	0.30	4.19	2.94	0.25	1.78
2.52	0.40	8.38	5.92	0.35	2.24
3.18	0.50	16.8	11.8	0.45	2.82
4.00	0.60	33.5	23.8	0.55	3.57
5.04	0.70	67.0	47.4	0.65	4.49
6.34	0.80	134	94.7	0.75	5.66
8.00	0.90	268	189	0.85	7.12
10.1	1.00	536	379	0.95	8.98
12.7	1.10	1.07×10^3	758	1.05	11.3
16.0	1.20	2.15×10^3	1.52×10^3	1.15	14.3
20.2	1.30	4.29×10^3	3.03×10^3	1.25	18.0
25.4	1.40	8.58×10^3	6.07×10^3	1.35	22.6
32.0	1.50	17.2×10^3	12.1×10^3	1.45	28.5
40.3	1.60	34.3×10^3	24.3×10^3	1.55	35.9
50.8	1.71	68.7×10^3	48.6×10^3	1.66	45.3
64.0	1.81	137×10^3	97.1×10^3	1.76	57.0
80.6	1.91	275×10^3	194×10^3	1.86	71.9
102	2.01	549×10^3	388×10^3	1.96	90.5
128	2.11	1.10×10^6	777×10^3	2.06	114
161	2.21	2.20×10^6	1.56×10^6	2.16	144
203	2.31	4.40×10^6	3.11×10^6	2.26	181
256	2.41	8.79×10^6	6.22×10^6	2.36	228
322	2.51	17.6×10^6	12.4×10^6	2.46	287
404	2.61	35.1×10^6	24.9×10^6	2.56	361
512	2.71	70.3×10^6	49.6×10^6	2.66	452
646	2.81	141×10^6	99.4×10^6	2.76	573
812	2.91	281×10^6	199×10^6	2.86	724
1020	3.01	562×10^6	397×10^6	2.96	909

Fig. 5

of particle volume), or where measurement of relative volume is important (e.g., Parsons 1965). This is commonly used for blood-cell counts and can also be used for plankton cultures and larval counts.

Example: Set the sensitivity so that the pulses from the largest particles just reach to the top of the oscilloscope screen. Take counts over definite threshold intervals (e.g., 5–10, 10–15, etc.). These will correspond to definite ranges of particle volume. It is often convenient when making counts in this way to set a constant threshold "window". This is done simply by putting the upper threshold mode switch to LOCKED (See C.I(3)).

Logarithmic scale of particle diameter or volume: This is used where large range of particle size occurs, e.g., suspended matter in the sea (Sheldon and Parsons 1967). The most convenient, and indeed the only practical scale to use, is one such that particle volume varies by a factor of 2 or $2^{\frac{1}{2}}$ for each step. One such scale starting at a particle diameter of 1 μ is given in Fig. 5. This is also a log. scale of particle diameter. Scales based at other sizes can easily be calculated if required.

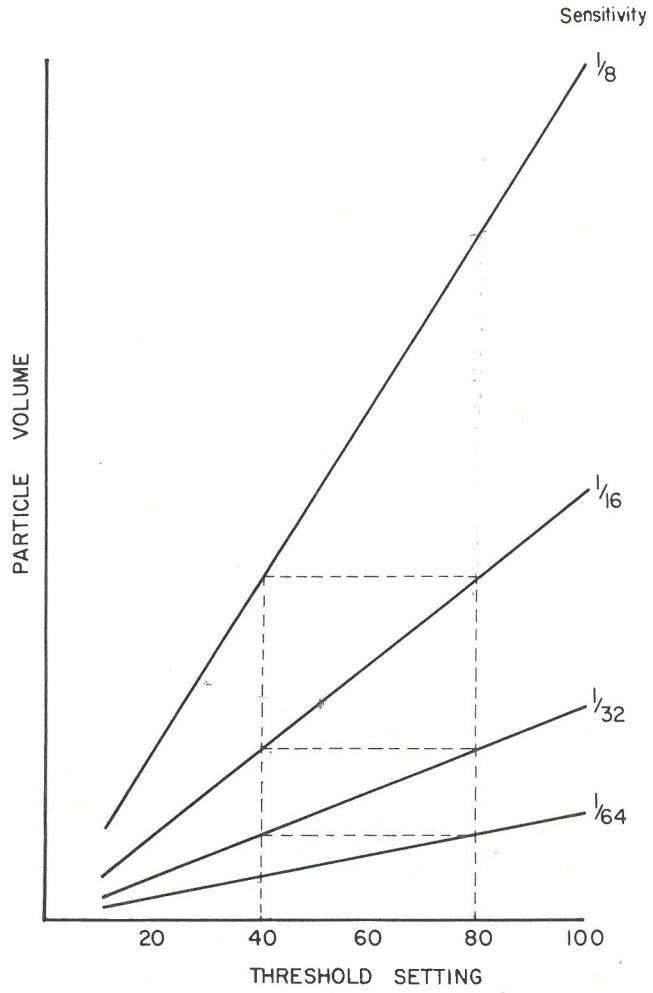


Fig. 6

Reference to Fig. 6 will show how such a scale can be used in practice. Assume that at sensitivity 1/32 particles of 2 μ diameter (4.19 μ^3 volume) correspond to a threshold setting of 40. Then particles of 2.52 μ diameter (8.38 μ^3 volume) correspond to a threshold

Vial 3 1.305 μm diam = 1.164 μm³ vol.

setting of 80. But 2.52 μ diameter particles also correspond to a threshold setting of 40 at sensitivity 1/16. Therefore, if the lower threshold is set at 40 and upper threshold is set at 80, then at sensitivity 1/32 particles with diameters between 2.0 μ and 2.52 μ will be counted. With the same threshold settings and sensitivity 1/16 particles 2.52 μ to 3.18 μ will be counted. It is clear, therefore, that counts can be made on a log. scale such that volume increases by a factor of 2 simply by setting lower threshold at some figure, upper threshold at twice this and taking counts at sensitivities such that each differs from next by a factor of 2.

$2^{1/2} = 1.4142$

Counts can be made at closer intervals by changing the sensitivity by a factor of $2^{\frac{1}{2}}$. In this case the upper threshold should be set at $2^{\frac{1}{2}}$ the value of lower threshold. Example: Set the lower threshold at 40 and the upper threshold at 56.5 (i.e., 40 x $2^{\frac{1}{2}}$). With the sensitivity at 1/16 (e.g., Amplification 1/4 Aperture Current 1/4) particles 2.52–2.82 μ will be counted. With the sensitivity at $\frac{2^{\frac{1}{2}}}{16}$ (e.g., Amplification 1/4 Aperture Current .354) particles 2.82–3.18 μ, etc. This scale is closely graded enough for the most exacting work.

Arithmetic scale of particle diameter: This is not a very convenient scale to use, but it might be needed for certain types of count, and particularly for comparison with microscope counts. The easiest way to write out the calibration is to plot the relationship between particle volume and instrument setting and then read off from this the instrument settings for each interval of particle diameter selected. It is also possible to calculate the instrument settings directly. It will be necessary to change instrument settings (sensitivity or thresholds or both) for each count, and great care should be taken to check the settings before each count. Figure 7 shows part of an actual calibration sheet for a 100 μ aperture. Note that for each diameter interval there is more than one possible instrument setting, e.g., counts between 11.5 μ and 12 μ diameter could be made at sensitivity 4, thresholds 32 and 36, or at sensitivity 2, thresholds 64 and 72. For most purposes the latter settings would be preferable.

(3) STABILITY

The response of the instrument depends on the difference between the resistivity of the particle and the resistivity of the electrolyte. It would appear, therefore, from purely theoretical considerations, that as the resistivity of an aqueous electrolyte varies with temperature and concentration of solute, the calibration should also vary with temperature, etc. This was the case with earlier models (model A) which ideally should be used under stable conditions of temperature, and should be calibrated for each electrolyte used. However, the model B Coulter Counter is designed so that calibration remains constant over a wide range of temperatures and electrolyte concentrations. In practice we have found that calibration remains constant between 5 C and 25 C using normal sea water (S ~ 30‰).

Calibration also remains constant over the range of salinity 8‰ – 40‰ (temp. 20C). For most applications in marine work using sea water as electrolyte a single calibration at 20°C with normal seawater will suffice for most purposes. However, if very high or very low salinities are encountered at the same time as high or low temperatures it would be wise to check the calibration.

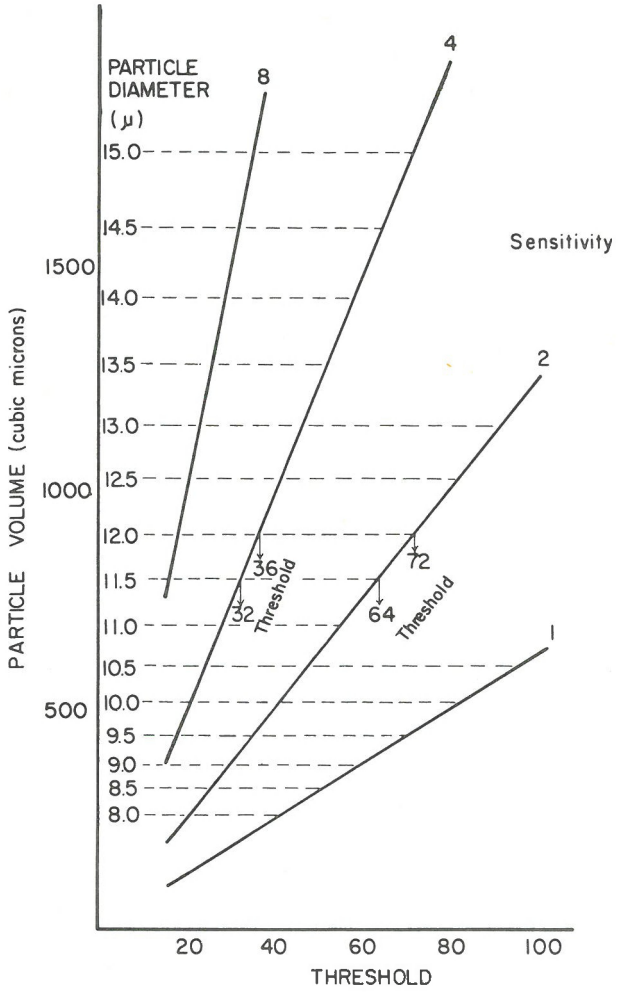


Fig. 7

IV. RELIABILITY

(1) ACCURACY

It is not easy to check absolutely the accuracy of counts and the measurement of particle size. This is partly due to the fact that other methods of counting and sizing either measure a different particle property (e.g., sedimentation rate) and are, therefore, not strictly comparable, or are to some extent subjective (e.g., haemacytometer). However, comparisons have been made of counts with the Coulter Counter and with other established methods (Maloney et al 1962) and there is little doubt that the instrument will count and size particles accurately, providing that certain precautions (see below) are taken. The measurement of density of sand grains (see B. V (1)) is a further indication that the instrument can count and size to a high degree of accuracy.

(2) PRECISION

It is self-evident that with small sub-samples or for suspensions with low particle concentrations, the number of particles counted will not be truly representative of the total sample. There will be random variations about some mean value. The precision which can be expected is shown in Fig. 8. The reproducibility of a count appears to be independent of aperture size, sample volume, or instrument sensitivity setting and depends only on the number of particles counted.

The following table will serve as a rough guide to the precision of count data (confidence limits given are two standard deviations (95%).

For a count of 2000 the true count is 2000 ± 65 (3.3%)
For a count of 200 the true count is 200 ± 12 (6%)
For a count of 70 the true count is 70 ± 11 (16%)
For a count of 30 the true count is 30 ± 8 (27%)

As larger samples are taken the reproducibility of count data improves until random variation becomes negligible. However, if large counts are due to high particle concentrations the accuracy of the count decreases, even though good reproducibility is maintained. This is because coincident passages occur. Two particles pass together and are counted as one. It is obvious that coincident passages will occur at all particle concentrations but at very low concentrations the probability of coincident passages is small and may be neglected. As particle concentration increases the proportion of coincident passages rapidly increases.

For simple distributions with fairly restricted range of particle size it is possible to count relatively concentrated suspensions and to make accurate estimates of coincident passages. Charts and graphs of corrections are included in the instrument instruction manual. Coincidence corrections can also be calculated from the following formulae:
The number of coincident passages $n_c = P\left(\frac{n_i}{1000}\right)^2$ where n_i is the observed count.

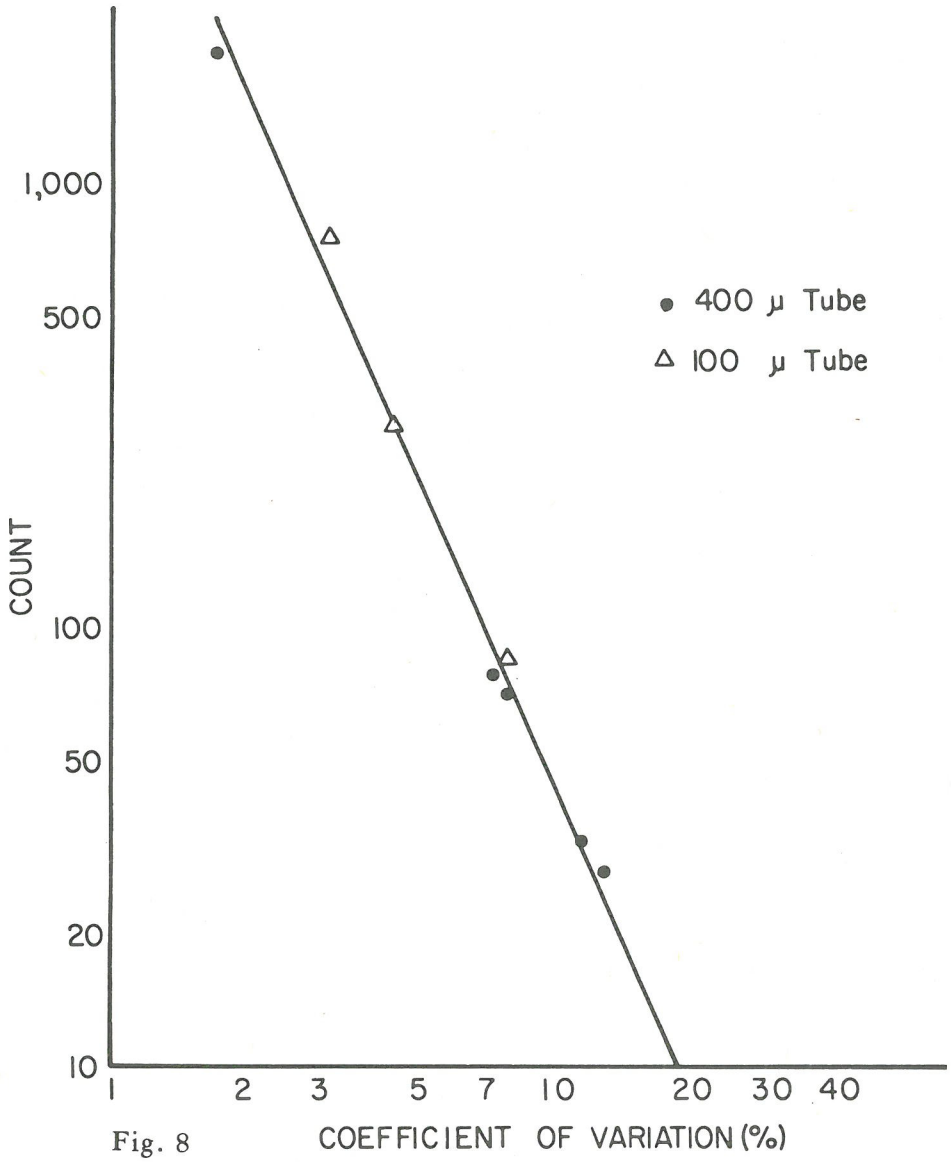


Fig. 8

The coincidence factor is given by $P = 2.5 \left(\frac{500}{V}\right) \left(\frac{D}{100}\right)^3$ D = aperture diameter in μ, V = sample volume in μ-litres

The true count is, therefore, $n_i + n_c$

This gives coincidence corrections accurate to about 1% for counts to 10% coincidence.

When particle distributions are encountered with a large range of particle size these simple corrections cannot be applied. It is not clear why this should be but we have found in practice that if high concentrations of particles with a large range of size are counted, accurate estimates of coincidence cannot be made. We have found that with most particulate systems encountered in marine work it is better to arrange that the particle concentration is such that if all particles in suspension are considered, coincident passages will not be more than 10%. To obtain an approximate check of particle concentration see A. III (2). Recommended maximum concentrations of particles are given in Fig. 9. With particle concentrations at this level the coincidence of counts taken between selected size limits can

usually be ignored. However, because of the low total concentration of particles the number of large particles in suspension may be small enough to seriously affect precision. This disadvantage can be overcome by taking larger sub-samples. There is no doubt that the Coulter Counter works best with relatively low particle concentrations, and concentrations should be arranged so that particles pass approximately one at a time and coincident passages are few.

With small apertures it is possible to approach the maximum counting rate of the instrument with suspensions giving less than 10% coincidence. It is always advisable with tubes smaller than 50 μ to check not only particle concentration but also the counting rate. This should not be greater than about 4–5000 counts per second.

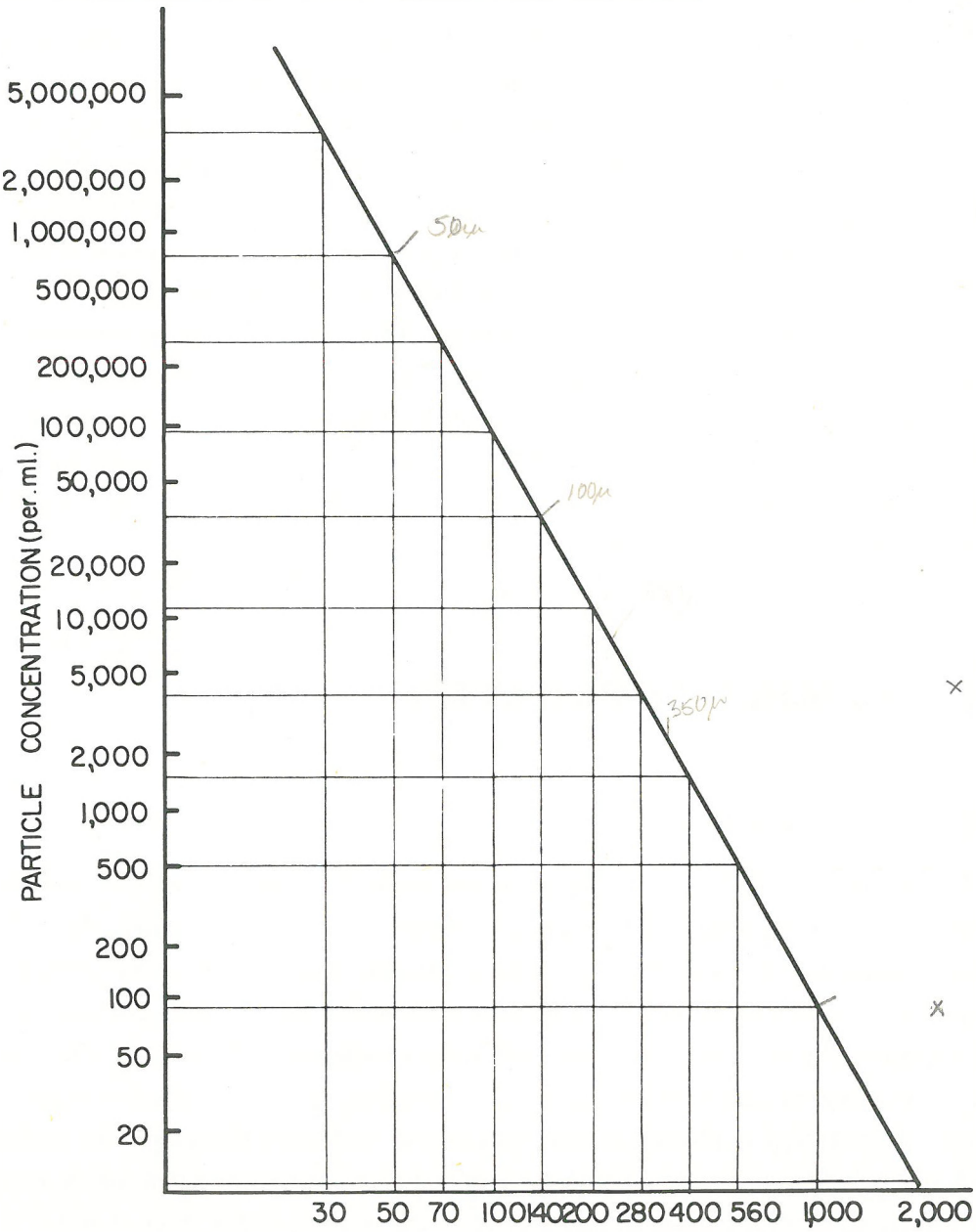


Fig. 9

(3) COUNTING SMALL PARTICLES WITH LARGE APERTURES.

When either discontinuous or closely graded distributions are measured with large apertures (greater than 400 μ) spurious counts may be obtained at small particle sizes.

With discontinuous distributions, particularly those with large numbers of particles near the upper limit of the range of an aperture and also large numbers near the lower limit, it is difficult to count small particles accurately. It seems that with large apertures the instrument cannot adequately resolve small and large pulses in close succession. However, with distributions of this type it is usually possible to separate the population of large particles on a screen and then measure the two populations separately, using different apertures if necessary.

With closely graded distributions, if integral counts are made (i.e., with lower threshold only) the count may increase as successively smaller particles are measured, then at some point will begin to decrease. This is clearly impossible. The decrease in count is due to inevitable limitations of the electronics and is related to the response time of certain of the components. The true count will be the largest count obtained. If differential counts are made (i.e., using both thresholds) the count at smaller sizes may increase. For samples with unknown size distributions it is difficult to decide whether this increase is spurious or whether it is due to a population of small particles. This difficulty can be resolved by changing to integral counts. If the count decreases at smaller sizes then the differential count is spurious, but if the count increases with decreasing particle size then a population of small particles is present. These should be screened off and counted separately. These difficulties are not found with continuous distributions covering a large range of particle size or with apertures smaller than 400 μ.

V. ACCESSORY APPARATUS

(1) THE MODEL J PLOTTER

The model J plotter can be attached to the model B counter to make an automatic graphical record – in the form of a histogram – of particle size distribution. The distribution by number is measured on an arithmetic scale of particle volume. The plotter takes approximately 110 seconds to record a complete distribution and as each subsample is counted automatically for a standard time, the volume sampled will vary with the size of the aperture. The count recorded by the plotter is, therefore, based on a relative scale 0 to 100. The particle distribution is plotted at intervals of four threshold settings. The actual number of particles in a selected interval can be determined from a separate count using the manometer to sample a definite volume of suspension. The number of particles in all other size

settings can then be obtained as a simple ratio of the height of the enumerated setting to the height of each of the other settings. Since the threshold settings may be calibrated in terms of volume, an integrated value for the total volume of the particulate material can be obtained. This is an advantage over count alone since increases in the volume of such particles as phytoplankton cells may often occur (e.g., with chain forming species) with little change in the total count of the sample. A discussion of the use of a model J plotter in measuring the growth rate of chain-forming phytoplankton will be found in Parsons (1965).

The precision of integrated volumes obtained with the model J plotter is partly a function of the precision of the model B counter and partly a function of the plotter itself. Since the plotter cannot be used independently of the model B counter the following examples of precision are a function of both plotter and counter reproducibility.

The only variable control on the model J plotter is an eight-step-sensitivity scale with a vernier adjustment. Each step up on the scale in a twofold multiple of the previous step.

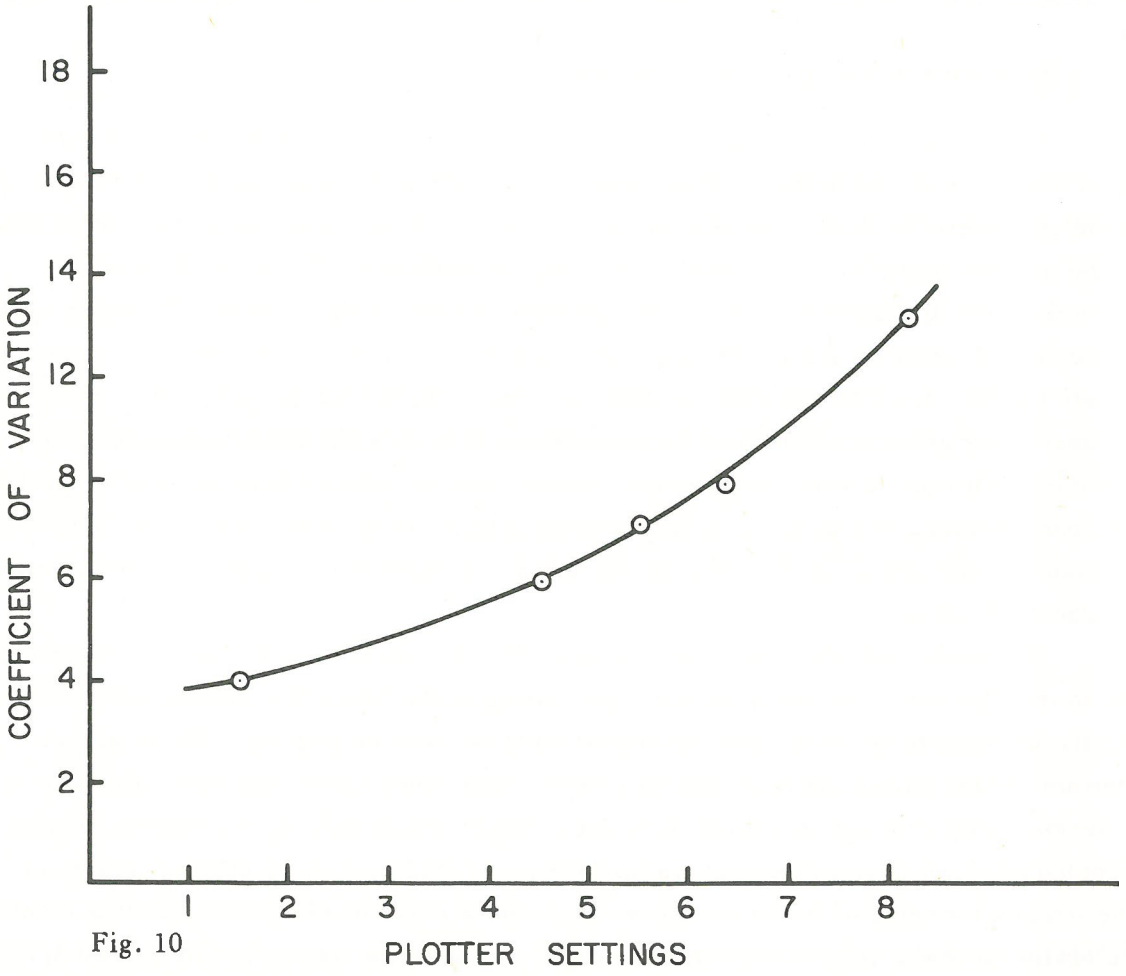


Fig. 10

Figure 10 shows the change in the coefficient of variation for five different steps on the sensitivity scale. From this figure it is apparent that the plotter should be used at low sensitivities by counting reasonable concentrations or particles, providing that concentrations are such that coincidence corrections are not too great.

(2) THE AUTOMATIC TIMER

It is necessary when using large apertures for the concentration of particles to be low in order to avoid excessive coincidence. Also, even with small apertures there are cases where the particle concentration can be very low (e.g., particulate detritus in the sea). The largest manometer volume (2 ml) is then not enough to give good reproducibility of the count, and it is necessary to use a timing device which takes over the function of the manometer. This is placed between the glassware unit and the amplifying and counting unit.

To operate, the counting unit is activated by drawing mercury back in the manometer in the normal way, but the control tap is left open so that the sample suspension is continuously drawn through the aperture by the pump. The counter is then switched on and off by the timer. Since the pump pressure is constant, the flow-rate of the sample will be constant, and, therefore, counts for a standard time will give a sample of standard (but unknown) volume. If it is necessary to know the sample size the flow-rate can be measured.

(3) THE MODEL M VOLUME CONVERTER

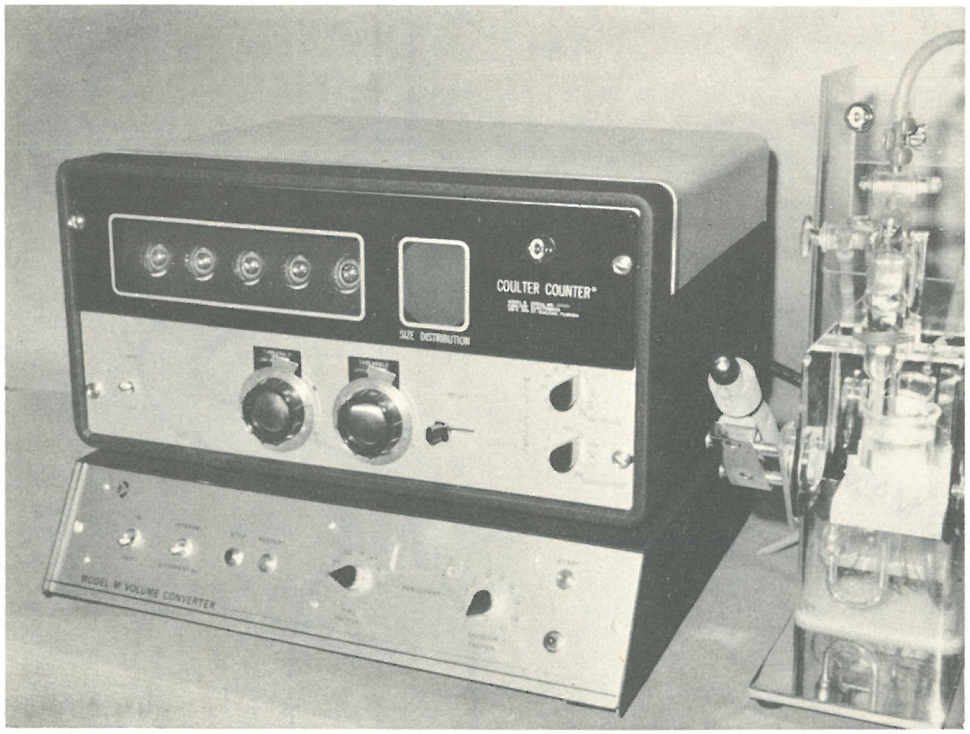
For certain types of particle population, (e.g., particulate detritus) count data should be given not as numbers of particles in a certain size range but as total volume of particles. Normally if data are required in this form it is necessary to multiply each count by the average particle volume for the size range considered. The model M volume converter does this automatically. The instrument gives results in terms of relative volume in which each grade is double the preceding grade (e.g., Fig. 5). In principle the instrument is very simple. If a particle count is made at some selected volume grade, then a count at the next lower grade would have to be twice as great to give the same total volume of particles. With the volume converter the second count is reduced by a factor of 2, so that the count obtained is a measure of total volume relative to the first count. This reduction in count is achieved either by reducing the volume of suspension sampled, or by reducing the count registered.

The method of operation is as follows: The threshold controls (of the counter) are set so that the upper control is at twice the setting of the lower (2 x volume difference) and the sensitivity is set so that the largest particles will be counted. The principal controls on the volume converter are an electric timer (time-factor selector) calibrated in seconds from 2 to 128 in powers of 2, and a device which reduces the number of counts recorded (division factor selector) graded from 1 to 1/512 also in successive powers of 2. The time factor selector is set to take a sample for a pre-determined time, (in this respect the operation of the volume converter is similar to that of the automatic timer, (see A.V(2)),

and the division factor selector is set at 1. The sample is taken and the number of particles is counted. The sensitivity is now changed by a factor of 2 so that smaller particles are measured – the threshold settings remaining the same – and one of the model M controls is also changed by a factor of 2. Either the division factor is changed to 1/2, so that two particles will be counted as one, or the time factor is moved to the next smaller setting so that only one half of the amount previously sampled will be taken. It is clear that under these conditions the count obtained will give a measure of total volume of particles relative to the first sample. With each sample, as the sensitivity is changed by a factor of 2, so the volume sampled or the number of particles counted is also changed by a factor of 2. The counts obtained are, therefore, measures of the relative volume of particles in each size grade. The instrument can be set to record either differential or integral distributions.

If an absolute measure of concentration is required, then the total volume of particles in one grade must be measured. This is done simply by taking a count on a known volume of sample and then multiplying the count by the mean particle volume for that grade.

When model M is attached to model B the instrument should be recalibrated. Once calibrated, providing that model M is not removed, there is no difference in calibration between using model B and model M together, or model B alone with model M switched off.



MODEL M VOLUME CONVERTER

(4) MODIFIED CONTROL PIECE FOR USE WITH LARGE APERTURES

When larger apertures (greater than 560 μ) are used on standard sampling units it is not possible to draw mercury back in the manometer. This is because considerable amounts of sample flow rapidly through large apertures and this overloads the pump so that it cannot maintain sufficient vacuum to draw back the mercury. Since the mercury cannot be drawn beyond beyond the start switch the counting unit cannot be activated.

Therefore, before large aperture tubes can be used it is necessary to modify the tap on the control piece (Fig. 11). In the position shown the pump will pull on the manometer only, so that the mercury can easily be drawn back, and the counter can be activated. Rotating clockwise by 120° will cause the pump to draw the sample, and a partial vacuum will be retained in the manometer. In this position samples can be counted using plotter, timer, or volume converter. Rotating a further 120° would allow the mercury to fall back in the manometer taking a sample through the aperture. A control piece modified for large apertures can, therefore, be used with small apertures and the manometer. However, with small apertures the standard control piece is more convenient to use.

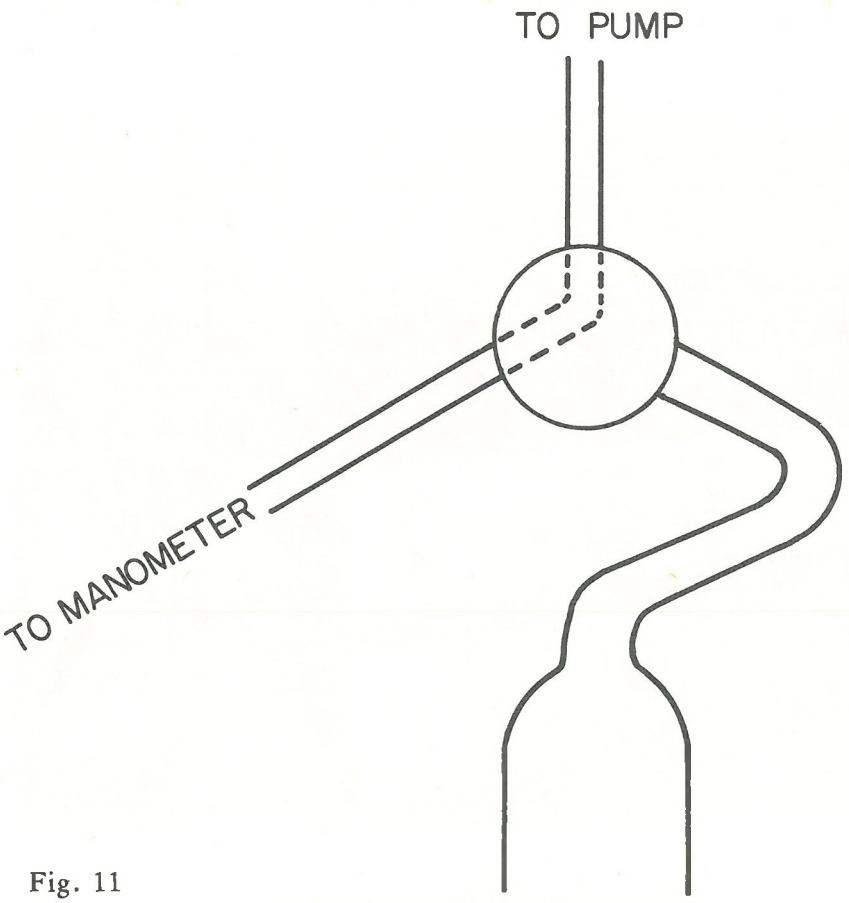


Fig. 11

SECTION B.

EXAMPLES

I. SUSPENDED MATTER IN SEAWATER

(1) INTRODUCTION

The Coulter Counter is ideally suited for measuring and counting particles suspended in seawater. Particle concentrations are often of the right order of magnitude so that neither dilution nor concentration is necessary. In these cases, samples can be taken from the sea and run directly through the counter.

Typically, there is a great range of particle size, and for some samples two or more apertures must be used. A logarithmic scale of particle size should be used (Sheldon and Parsons 1967).

It is often found that where a considerable range of particle size occurs particles fall into more or less discrete distributions. After a little experience in any one marine environment it is possible to select apertures so that most of the particles lie approximately in the middle of the range of each aperture.

The following examples have been selected to illustrate most of the characteristics found in particle distributions in temperature coastal waters.

(2) ESTUARINE SILT: size range 3-40 μ

This can be measured with a 100 μ aperture, but the first few samples of any series should be measured with a 200 μ aperture to check that there are few particles larger than 50 μ. A subsample should also be passed through a 37 μ screen and then counted with a 50 μ aperture to check that there are few particles small than 3 μ. It is necessary to screen the sample to be counted by the 50 μ tube in order to remove particles which would block the aperture (see C.I(4)). Particle concentrations can be very great and dilution of the sample by factors up to 100× might be necessary in many cases. The greater part of material in suspension in estuaries is of inorganic origin.

PROCEDURE

1. Take a water sample by any convenient method.
2. Put a 100 μ aperture on the counter and set the sensitivity to count the smallest particles.
3. Set the lower threshold at the lowest convenient level (usually in range 15–30).
4. Switch off the upper threshold.
5. Count 1/2 ml. All particles within the range of the tube will be counted.

6. If the particle concentration is greater than about 80,000 per ml, dilute until this concentration, (or less) is achieved – record the amount of dilution.
7. Start counting at the largest size range and make counts on a log. scale of particle size. Either the manometer or volume converter can be used. If the manometer is used make 2 ml counts at the larger sizes (to 20 μ), then 1/2 ml counts at the smaller sizes (20 μ – 3 μ).
8. Calculate the concentration of each size interval by multiplying the count by the mean particle volume for the interval.

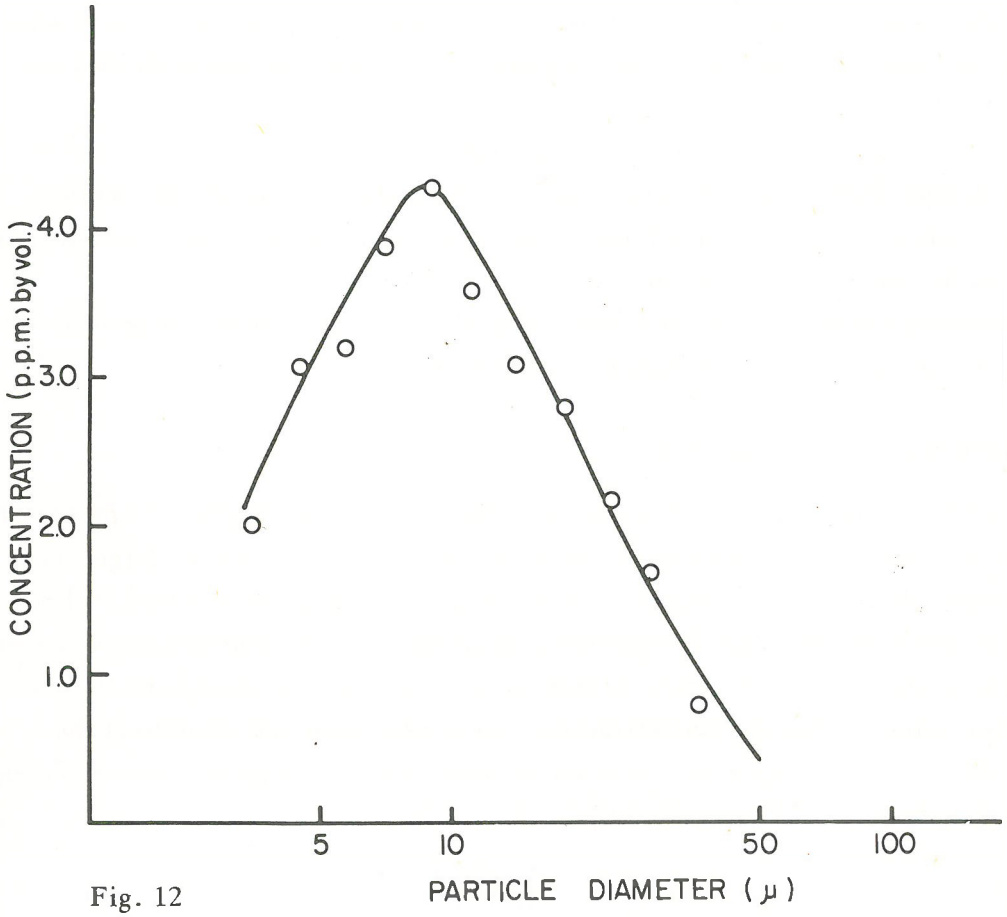


Fig. 12

Example: Fig. 12 gives a particle size distribution for estuarine silt. The material consisted largely of flocculated masses of very small inorganic particles; the principal constituent being quartz. Since the sample was not pre-treated in any way prior to counting (except for dilution) the figure probably gives a true representation of the grain size distribution of suspended matter as it actually existed in the estuary.

(3) PHYTOPLANKTON AND ORGANIC DETRITUS IN A COASTAL ENVIRONMENT: size range 6-250 μ.

In open coastal areas, there is rarely much mineral material in suspension, particularly if the coast is rocky. Suspended matter consists mainly of phytoplankton and organic debris, the debris being probably of both marine and terrestrial origin. The size of this material falls mainly in the range 10–250 μ, and the size distribution can be measured using two tubes, 560 μ and 200 μ. For these measurements an automatic timer (or model M volume converter) is essential.

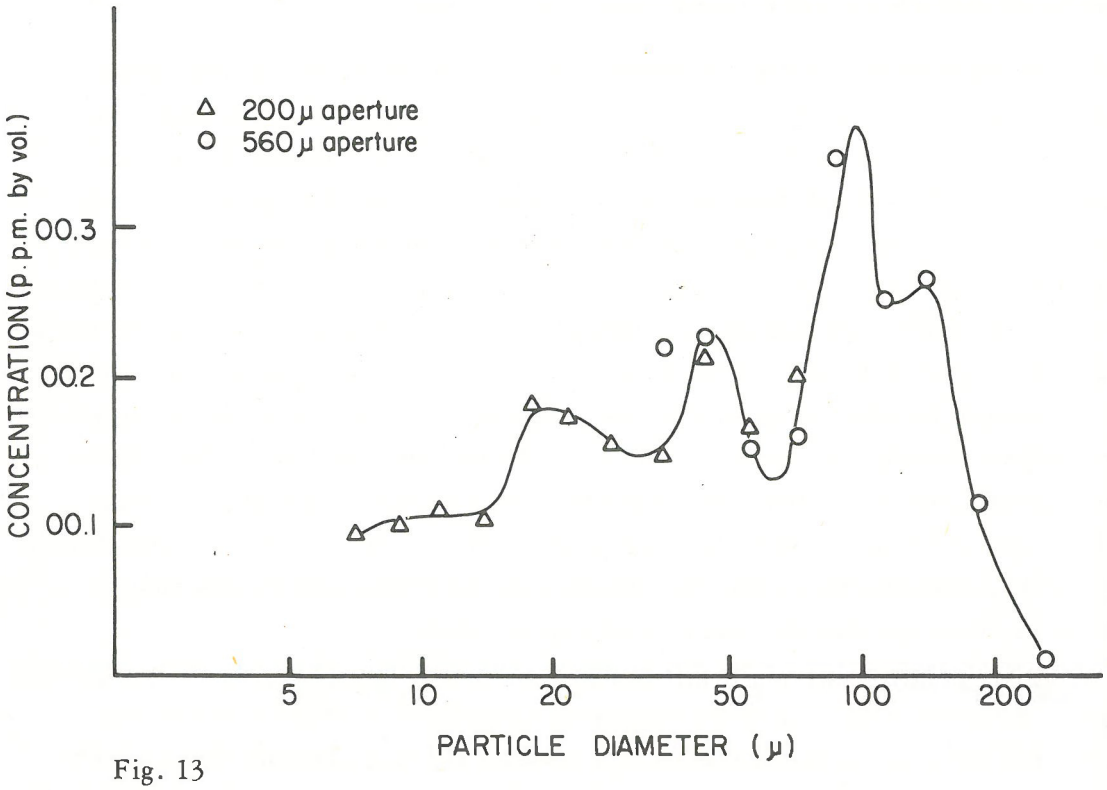


Fig. 13

PROCEDURE

1. Pour the samples into beakers carefully and leave to stand for a few minutes before measuring. Bubbles seem to form fairly readily in the size range 50-100 μ but not at other sizes. Agitate gently before measuring, or preferably, stir gently while measuring.
2. With a 560 μ aperture and an automatic timer make measurements from 250 μ to smaller sizes. At large sizes considerable volumes of sample may be needed (often 100-500ml) to obtain adequate counts.

3. When the limit of the 560 μ aperture is reached (about 20 μ) change to 200 μ. Screen the sample at 250 μ and count from 80 μ downwards. Some blockage may occur but it is not usually troublesome.
4. Measurements can be continued to about 6 μ.
5. Calculate results as in B.I(2).

Example: Figure 13 gives a size distribution of particles in water from a rocky coastal area. Note how this distribution differs from that of an estuarine environment. Particles are larger but the concentration of material in suspension is much less.

(4) COMPLETE PARTICLE SPECTRUM: size range 3-1000 μ

The procedure for measuring suspended particulate material over the size range 3-1000 μ is basically similar to that described in the previous section for size ranges of 6-250 μ. It differs, however, in that it is advisable to concentrate particles larger than 100 μ in order to avoid having to draw an excessive amount of fluid through the aperture.

For particles in the size range 200-1000 μ the extent to which samples have to be concentrated will vary with the environment, but thousandfold concentrations will generally be necessary. The 2000 μ aperture draws approximately 15 ml per second, so that the initial total volume of concentrated sample must be at least 1-2 litres. It is generally not practical to obtain this amount by filtering water samples collected in bottles, but a pumped sample of sea water strained through a screen will produce the desired concentration without undue delay. In practice it has been found convenient to use a zooplankton sample collected quantitatively with a 300 μ net, and dilute this to the desired volume. A second difficulty in obtaining 3-1000 μ size spectra is that, the operation of the 2000 μ tube is less easy to follow on the oscilloscope than the operation of smaller tubes.

The particular advantage of employing the type of continuous size spectrum suggested here is that it gives equal spacing to the traditional classification of nano-, micro- and macroplankton (if the scale could be continued, ultranano and megaplankton would also be included). Thus, if the definitions of the size of plankton groups are taken as nanoplankton (2 to 20 μ diameter), microplankton (20-200 μ diameter), macroplankton 200-2000 μ diameter) following Dussart (1965), then on the grade scale shown in Fig. 5 and in the example, Fig. 14, these plankton groups are given equal importance. (For further discussion, see Sheldon and Parsons, 1967.)

PROCEDURE

1. Collect a quantitative zooplankton sample with a net (pore size 300 μ) from the area of study. Dilute the sample to 2 litres and filter it through a 3 mm pore diameter sieve in order to remove the larger zooplankters. The concentration of particles should now be adjusted so that the final concentration in the size range 200-1000 μ is about 5 per ml. This can be readily checked by a preliminary macroscopic count of 10 ml of sample

before starting the size spectrum analysis. Using a 2000 μ aperture determine the distribution of particles over the size range ca 200-1000 μ following the same procedure described in B.I(3) for the use of the 560 μ aperture. (Due to the large volumes of sample involved, the 300 ml sample beaker should be replaced with a suitable reservoir of about 1000 ml capacity. Vigorous stirring is necessary to keep the particles suspended in this larger container.)

2. Concentrate the particulate material from 20 litres of seawater by straining through a 40 μ pore size sieve, and resuspend the particles retained on the sieve by back washing with 1 litre of seawater. Using a 400 or 560 μ aperture, determine the particle size distribution for particles over the size range ca 60-200 μ diameter as in section B.I(3), correcting the final volume of particulate material for the extent to which it has been concentrated.
3. Follow procedure described in B.I(2) but use a 100 μ aperture to determine particle size distributions over the size range, ca 3-30 μ diameter.

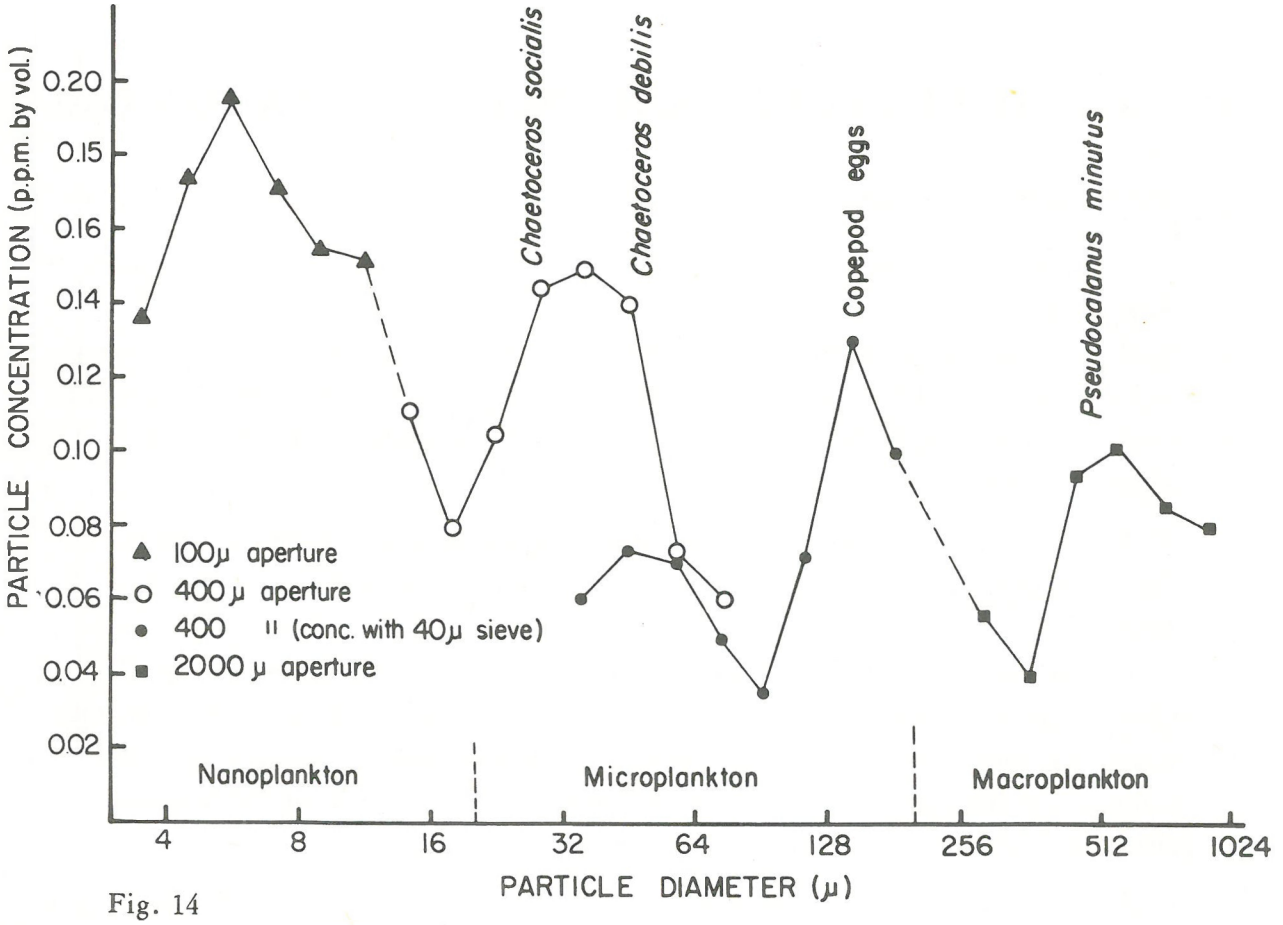


Fig. 14

Example: Figure 14 (taken in part from Sheldon and Parsons 1967) shows the result of a size spectrum analysis carried out as described above. The range over which the three different sized apertures were actually employed is indicated on the figure. Since there is always a region of overlap in determining particle counts with different aperture the actual range over which any one aperture is employed is in practice usually determined by keeping the counting time for individual samples down to relatively short periods (i.e., seconds rather than minutes) in order to avoid unnecessary delays. Thus, in Fig. 14 the 100 μ aperture was used for particle sizes ca 3-10 μ; the 400 μ aperture for particle sizes ca 10-200 μ, and the 2000 μ aperture for particle sizes ca 250-1000 μ. The effect of concentrating the sample with a 40 μ sieve is shown in the center of the microplankton region of the spectrum. The first two measurements (between 30-50 μ), made with the concentrated sample show that much of the material in this size range had been lost through the sieve but that there was good agreement with the unconcentrated sample for two measurements which overlapped in the region 50-80 μ.

II. ALGAL CULTURES AND PHYTOPLANKTON GROWTH

(1) DISTINGUISHING ALGAL SPECIES

It is possible to recognize from size frequency distributions individual species in mixtures of algae.

Example: In Fig. 15 distributions for 3 separate unialgal cultures are shown. These were all measured with 100 μ tube. The counter was calibrated to give an arithmetic scale of particle diameter (see A. III (2)) and these results are, therefore, comparable with determinations with a microscope.

The distributions of Monochrysis and Amphidinium are approximately normal but the distribution of Dunaliella is asymmetrical because of large numbers of old cells in the population.

A frequency distribution of a mixture of these cultures is also shown in Fig. 15. Amphidinium and Monochrysis can be easily recognized but Dunaliella has been nearly lost in the left hand side of the Amphidinium curve. The relative degree of resolution of the Amphidinium and Dunaliella populations would depend, to some extent, on the relative proportions in which they occurred.

Under natural conditions separate populations of the small algae could be recognized if the size difference was greater than 6 μ. For differences between 6 μ and 3 μ resolution of separate populations would depend on the relative proportions in which they occurred. Populations differing by less than 3 μ would be virtually impossible to separate on the basis of cell diameter.

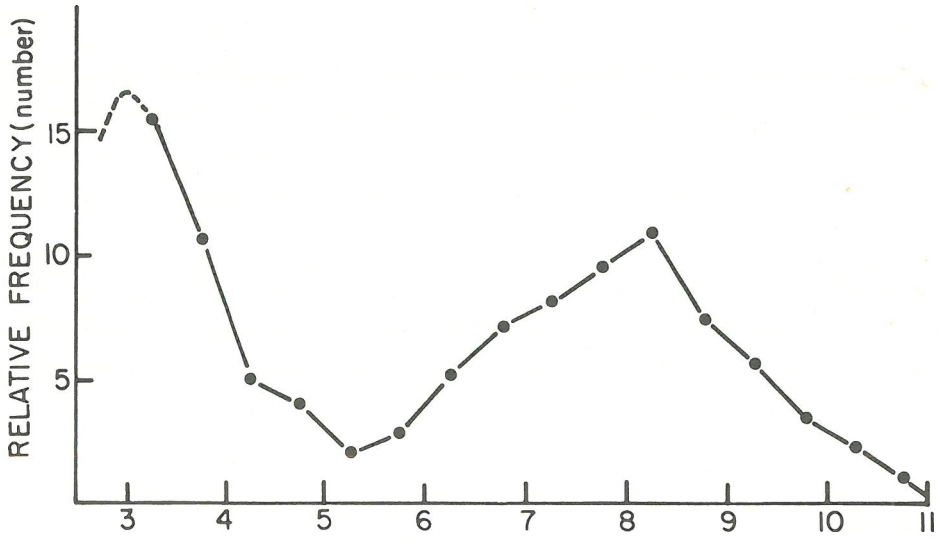


Fig. 15 A

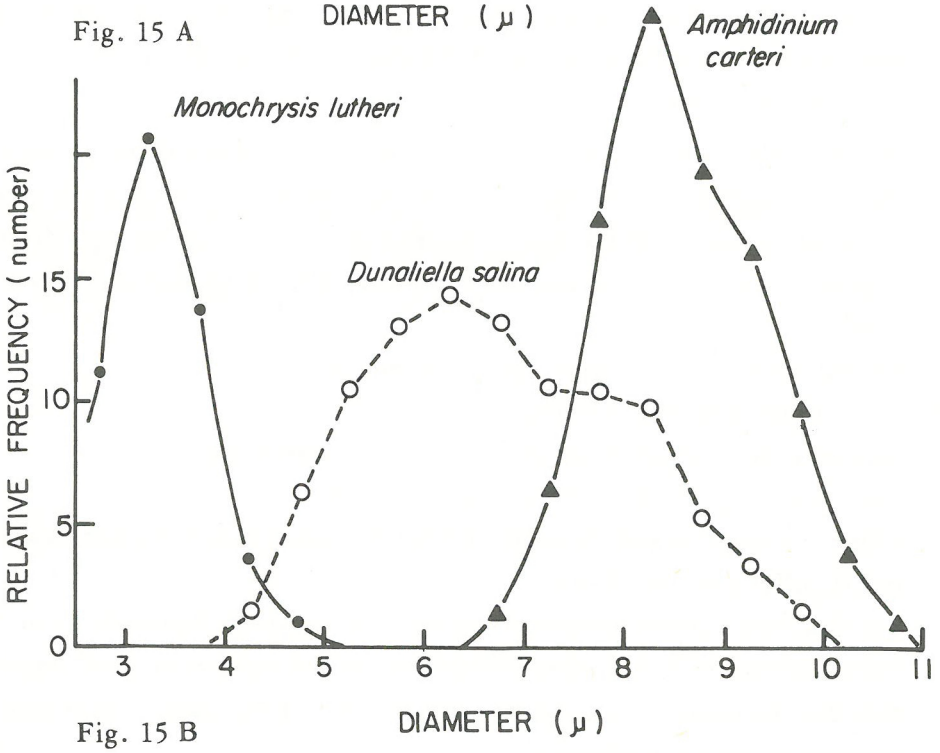


Fig. 15 B

(2) GROWTH OF UNIALGAL CULTURES

The use of the Coulter Counter for determining the growth constant *of phytoplankton cultures depends to some extent on the species being studied. For unicellular algae it is only necessary to determine the number of cells at the beginning and end of a known time interval. This can be done without calibrating the counter in terms of particle volume and the method involved is essentially the same as for the enumeration of red-blood cells (see Manual for model B Coulter Counters; also El-Sayed and Lee 1963 or Maloney et al 1962).

A more precise measurement of the growth constants of unialgal cultures is obtained, however, if the volume of the individual cells is considered as well as their number. To do this the instrument has to be calibrated as in A. III (2). For chain-forming species it is absolutely necessary to consider the volume increase of individual chains in order to determine the growth constant.

For the following procedures it is assumed that all sea water samples or culture media are in the salinity range (8-40‰). For samples outside this range the instrument should be recalibrated at a salinity close to that of the natural samples (see A. III (3)).

PROCEDURES

(a) Simple counts

1. Withdraw several ml of culture from a flask and determine the amplification and aperture current settings at which the pulses appear about half way up the oscilloscope screen.
2. Set the lower threshold above the electronic background noise as seen on the oscilloscope screen, and count the number of cells in a known volume of culture medium. If the count obtained is too great for the aperture tube employed (see Fig. 9), dilute the culture with fresh medium and repeat the count.
3. Repeat count (N_2) at time t_2 and determine k.

(b) Total cell volume and size distribution

1. Calibrate the instrument as described in section A. III (2).
2. Carry out the procedure above, steps 1 and 2.
3. Place the upper threshold at 40 and put the upper threshold mode switch in LOCKED position (C.I(3)).
4. Starting from a lower threshold setting of 4, move the threshold up at 4 threshold intervals, counting a known volume of medium at each interval.
5. Multiply the counts obtained for each interval by the mean cell volume represented by each interval.

*The growth constant is defined as $k_{10}\ (\text{days})^{-1} = \frac{\log N_2 - \log N_1}{t_2 - t_1}$ where N_1 and N_2 are the numbers of cells or volumes of particulate material at time t_1 and t_2.

6. Plot the results (cell volumes vs size) on arithmetic graph paper, and total the volumes (N_1) in each size category making up the normal distribution of cell sizes as seen from the plotted results.
7. Repeat cell volume determination (N_2) at time t_2 and determine k.

Note: If a model J cell size distribution plotter is available, the enumeration and plotting of the cell sizes is carried out automatically,

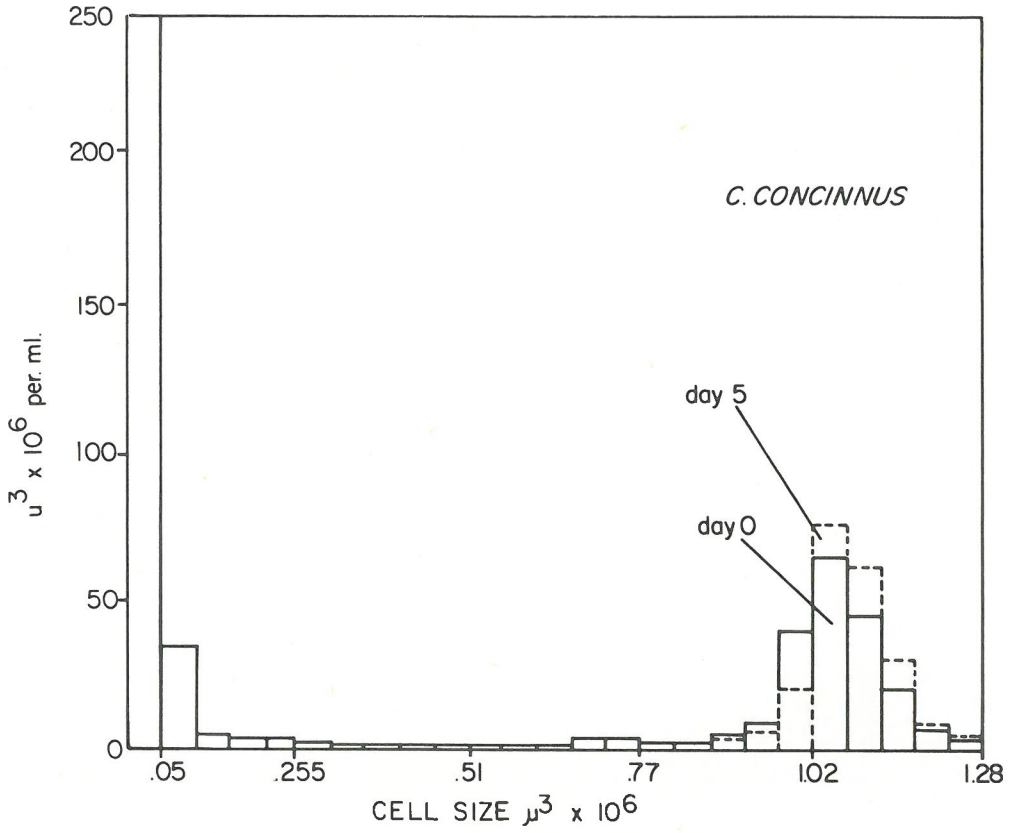


Fig. 16

Example: The example shown in Fig. 16 has been taken from Parsons, 1965 (Limnol. Oceanogr. 10: 598-602). The distribution of Coscinodiscus concinnus cells is shown at two times (day 0 and 5) as a function of cell volume.

(c) Chain-forming phytoplankton

1. The size distribution of chain lengths of a chain-forming phytoplankton species approaches a log-normal distribution. The growth rate and distribution can be determined as described below (B. II (3) (a)) or as described above (B. II (2) (b)). If the latter method is chosen, however, it is not possible to see the culture on the oscilloscope as a discrete population. The choice of amplification and aperture current settings is

best determined by selecting settings which place the volume of one cell in the phytoplankton chain * in the second or third thresholds 4-8, or 8-12 size categories. This allows for chain lengths of up to about 25 cells to be plotted on one arithmetic scale. If the chain length starts to exceed 25 cells/chain, the amplification or aperture current can be raised one setting to allow for up to 50 cells per chain to be plotted on the same scale.

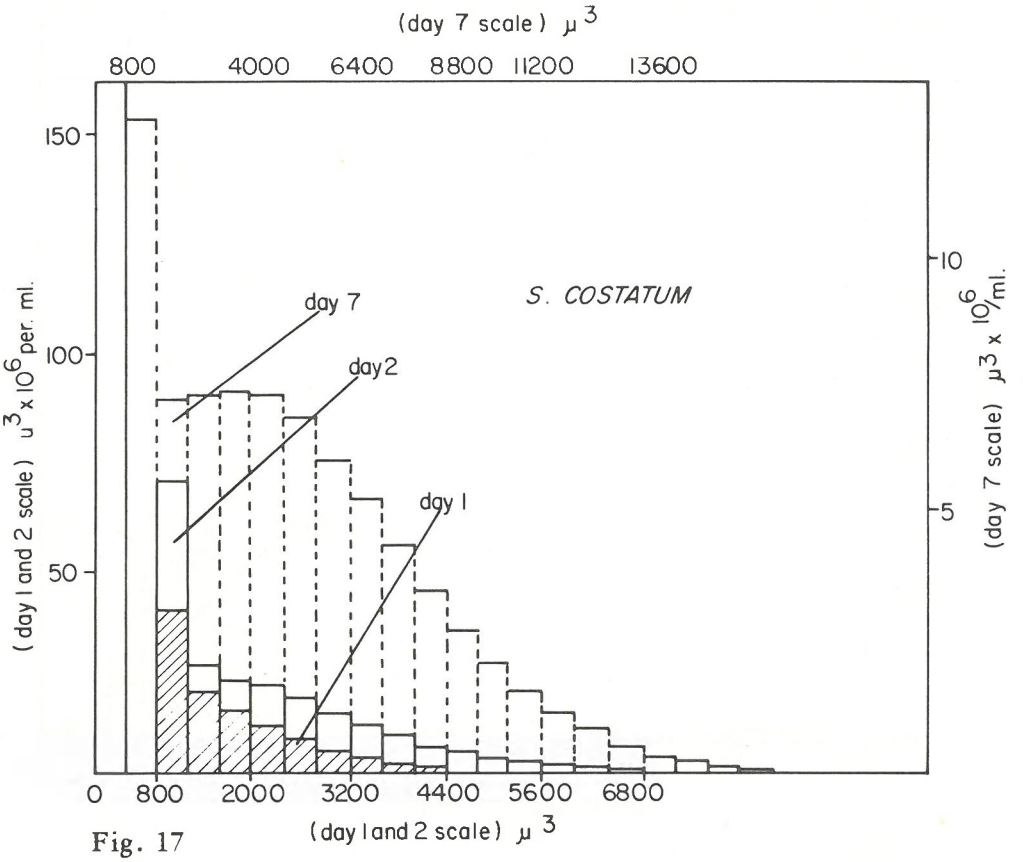


Fig. 17

Example: The example shown in Fig. 17 has been taken from Parsons, 1965 (Limnol. Oceanogr. 10: 598-602). The distribution of Skeletonema costatum plotted with a model J cell size distribution plotter, is shown at three time intervals.

(3) GROWTH OF NATURAL PHYTOPLANKTON POPULATIONS

(a) In the presence of small amounts of detritus

For natural populations it is preferable to describe particle sizes and growth constants in terms of a logarithmetic distribution. Since natural samples of sea water contain large quantities of detritus, the particle distributions obtained, and the growth constants after incubation, will represent the entire spectrum of particulate material (i.e., phytoplankton and

*The volume of one cell in a phytoplankton chain can be approximated from microscopic measurements.

detritus). Depending largely on what the information is required for, the growth of the entire spectrum of particulate material, or of a specific part of it (as shown below), or of only the phytoplankton may be determined.

PROCEDURE

1. Obtain seawater samples from desired depths. Divide each sample into two one-litre capacity glass bottles, one of which is completely darkened with a suitable paint, tape or cloth.
2. Stopper bottles and incubate a dark and light bottle, either at different depths in situ, or in a standard light incubator such as is used for C-14 incubation.

Fig. 18 Growth Rates of Suspended Particulate Material

Location: Saanich Inlet, B.C.
Date: 20/6/66
Incubation: 24 hr in situ

Diameter (μ) / Depth (m)		0		5		10	
		Coulter Counter Volumes (cu μ x 10^6/ml)					
		Dark	Light	Dark	Light	Dark	Light
Nanoplankton Region	3.57	0.10	0.10	0.38	0.21	0.19	0.15
	4.49	0.11	0.13	0.26	0.24	0.13	0.16
	5.66	0.16	0.20	0.28	0.27	0.20	0.20
	7.12	0.40	0.41	0.36	0.54	0.38	0.36
	8.98	0.46	0.50	0.53	0.51	0.53	0.63
	11.3	0.45	0.52	0.82	0.70	0.72	0.70
	14.3	0.92	1.14	1.64	1.50	1.26	1.15
	18.0	1.67	2.45	2.15	2.10	1.70	1.72
Microplankton Region	22.6	2.16	3.75	1.40	2.64	1.98	1.96
	28.5	2.35	2.60	1.14	2.07	1.58	1.86
	35.9	4.70	5.60	1.08	1.76	2.03	1.76
	45.3	2.48	3.36	0.57	0.84	1.22	1.36
	57.0	1.05	1.37	0.45	0.76	0.66	0.59
	71.9	0.51	0.79	0.37	0.74	0.38	0.41
	90.5	0.44	0.49	0.16	0.22	0.16	0.14
Total		17.96	23.41	11.59	15.10	13.12	13.15
Growth const. ($Days^{-1}$)		0.114		0.115		no growth	
Nanoplankton total		4.27	5.45	6.42	6.07	5.11	5.07
Growth const. ($Days^{-1}$)		0.106		no growth		no growth	
Microplankton total		13.69	17.96	5.17	9.03	8.01	8.08
Growth const. ($Days^{-1}$)		0.118		0.242		no growth	

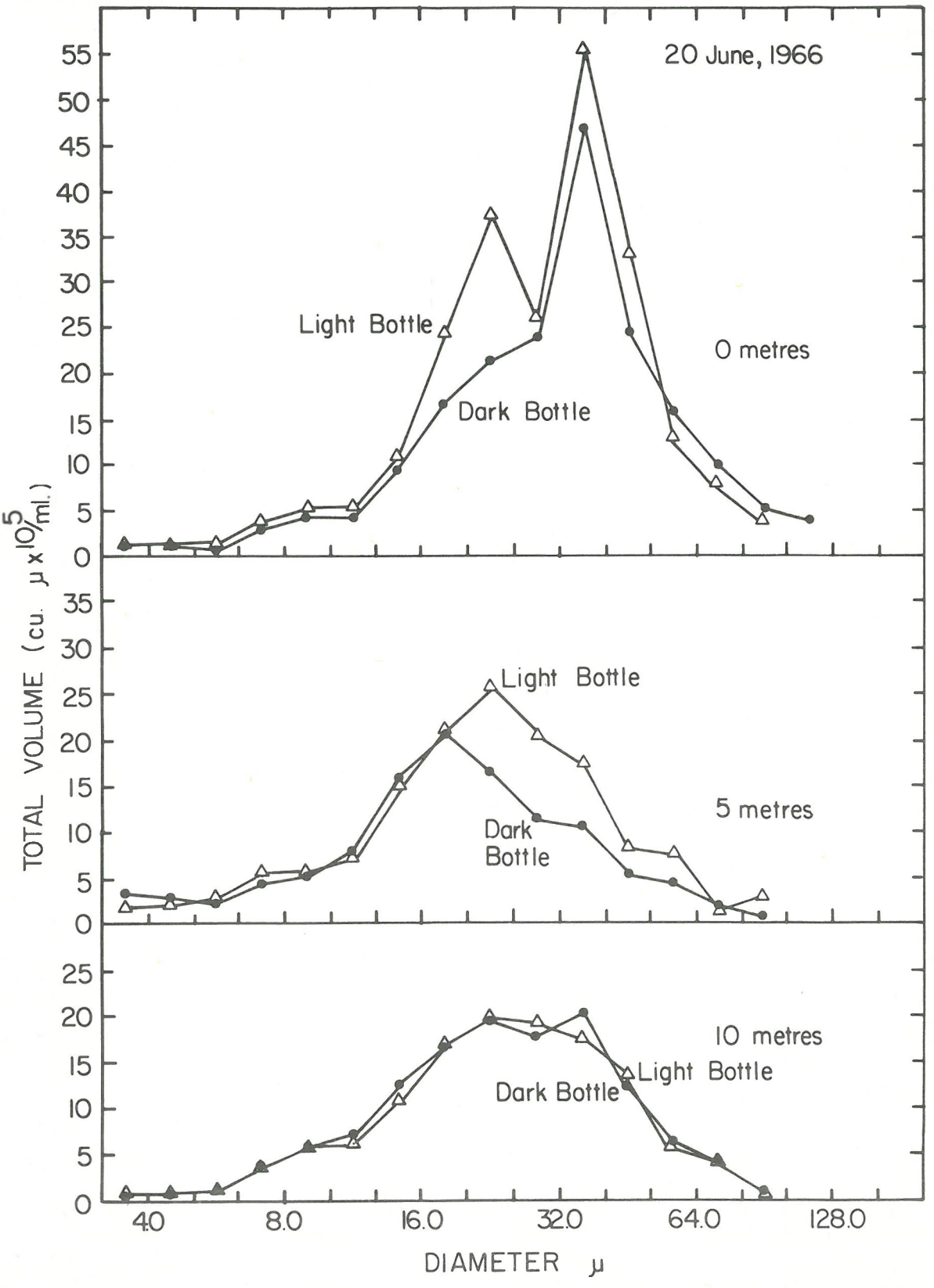

20 June, 1966
Light Bottle
Dark Bottle
0 metres
Light Bottle
Dark Bottle
5 metres
Light Bottle
Dark Bottle
10 metres
TOTAL VOLUME (cu. μ x 10^5/ml.)
DIAMETER μ
4.0
8.0
16.0
32.0
64.0
128.0

Fig. 19

3. After 24 hours, measure the spectrum of particulate material as described in section B. I (3).

4. Note: Slightly different results are obtained in this procedure if the spectrum of the initial seawater sample is compared with the 24 hour light bottle sample instead of using the 24 hour dark bottle sample. These differences are partly due to real changes in the spectrum of particulate material during 24 hours in the dark and partly to the effect of shaking to insure a uniform suspension of particles after 24 hours incubation.

Example: Figure 18 shows the result of a light and dark bottle incubation of sea water from three depths. These results are plotted in Fig. 19. From Fig. 18 it may be seen that the growth constant of the total particulate material at 0 and 5 m was about the same, but that at 10 m there was no significant growth. The principal difference between the 0 and 5 m samples is shown to occur in the growth rate of different portions of the spectrum. Thus, in the 0 m sample appreciable growth occurred in the nanoplankton region of the spectrum whereas no growth occurred in this region at 5 m. The growth of microplankton at the latter depth, however, was about twice the growth rate of the microplankton at 0 m.

(b) With considerable detritus

In the previous section the growth rate of the entire spectrum of particulate material or of a certain size fraction, was obtained as a relative measure of the growth activity of the suspended material in sea water. These values can be easily obtained from two measurements of the size spectrum of particulate material and they approximate the true phytoplankton growth-constant when the ratio of biomass of plankton to detritus is large (e.g., 5 to 1). Cushing and Nicholson (1966) have described a method for determining the growth constant of phytoplankton corrected for the amount of non-living particulate material. This involves several determinations of the total volume of particulate material, and an additional calculation to determine the quantity of detritus, (see C.I(5)).

PROCEDURE

1. Measure the size spectrum of suspended material in sea water as described in section B. I (3).

2. Incubate a 1-litre sample in a glass stoppered bottle either at the depth from which the sample was taken or in a light incubator such as is employed in C-14 photosynthetic studies.

3. Remeasure the size spectrum of particulate material after successive time periods (e.g., at 12 hour intervals).

4. Determine the total volume of material produced at different time intervals. Calculate $\hat{k}$ (the apparent growth constant) by subtracting different values of $\hat{D}$ (the estimated volume of detritus) from the total volume of material produced at different time intervals. The value of $\hat{D}$ for which $\hat{k}$ does not change with time is then used to correct the measured particle volumes to give the true phytoplankton volumes at different times.

Fig. 20 Growth Rate of a Natural Phytoplankton Population

Location: Departure Bay
Date: 24/12/66
Period of Incubation: 72 hours
Continuous illumination

Diameter (μ) / Time intervals (hr)	0	24	48	72
	Coulter Counter Volumes (cu μ x 10^4/ml)			
7.12	3.9	6.3	14.0	34
8.98	5.0	8.5	32.0	91
11.3	4.0	7.5	32.5	106
14.3	3.8	6.1	17.2	52
18.0	3.3	4.4	5.8	15
22.6	3.1	5.0	3.5	6.2
28.5	2.5	5.0	5.8	9.8
35.9	4.4	5.1	11.0	26
45.3	0.7	4.2	10.0	35
57.0			1.8	20
71.9				18
90.5				5.4
Total	31	52	134	438

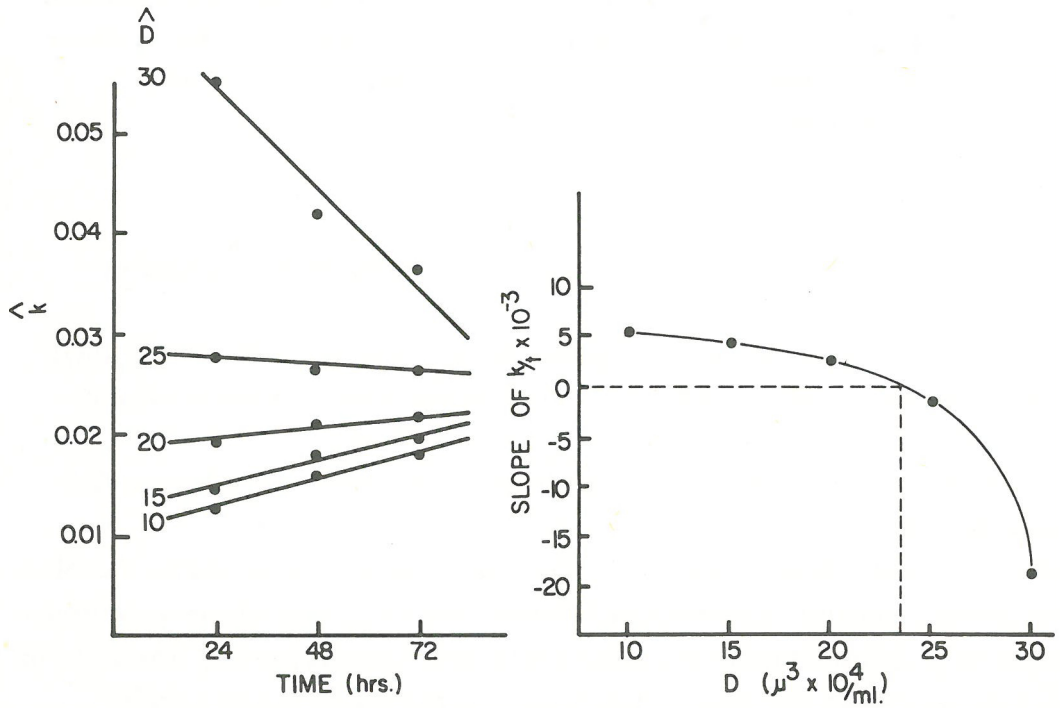


Fig. 21

Example: In the following example a sample of sea water was collected during the winter from Departure Bay, British Columbia and incubated at 10° C for several days. After an initial lag phase a small population of phytoplankton developed and the growth of this population was followed for the next 72 hours. The results are shown in Fig. 20. The calculation of the true growth constant of this population is also shown in Fig. 21 and as follows:

Calculation of $\hat{k}$

For $V_o = 31 \times 10^4$ $V = 52 \times 10^4$ $t = 24$

$\hat{D}$	10	15	20	25	30	($\times 10^4$)
$V - \hat{D}$	42	37	32	27	22	
$V_o - \hat{D}$	21	16	11	6	1	
$\frac{V - \hat{D}}{V_o - \hat{D}}$	2	2.3	2.9	4.5	22	
$k = \frac{V - D}{V_o - D} / t$	0.0126	0.0148	0.0191	0.0274	0.0555	

Similarly for $V = 134 \times 10^4$

$\hat{D}$	10	15	20	25	30	($\times 10^4$)
$\hat{k}$	0.0160	0.0182	0.0213	0.026	0.042	

and for $V = 438 \times 10^4$

$\hat{D}$	10	15	20	25	30
$\hat{k}$	0.0182	0.0195	0.0216	0.0256	0.0365

The values of $\hat{k}$ at different times for different values of $\hat{D}$ are plotted in Fig. 20. The slopes of the lines are determined for:

$\hat{D}$	10	15	20	25	30	($\times 10^4$) and the

slopes of $\hat{k}$ on t for 72 hours unit time are:

0.0056 0.0047 0.0025 -0.0018 -0.019

The value of $\hat{D}$ at which there is no change in $\hat{k}$ with time is 2.35×10^4 μ^3/ml. The volume of detritus is, therefore, 23.5×10^4 μ^3/ml, and the original phytoplankton volume (V_o) is 7.5×10^4 μ^3/ml. (i.e., $31.0 - 23.5$ μ^3/ml).

The growth constant k_{10} $(\text{hours})^{-1}$ is $\frac{\log (134\text{-}23.5) - \log .7.5}{48} = .0245$

$\therefore$ The generation time is $\frac{0.3}{0.0245} = 12.2$ hours.

From Fig. 20 it may be seen that the population of phytoplankton developed two peaks, one at 11.3 μ diameter and the other at 45.3 μ diameter. The growth constant for the total population was estimated. As with the previous procedure (Fig. 18) it is possible to obtain separate growth constants for the two different phytoplankton populations shown in Fig. 19 (see C.I(6)).

III. ZOOPLANKTON GRAZING

The Coulter Counter can be used to measure the effect of zooplankton grazing on a phytoplankton population. If the phytoplankton are part of a unialgal culture, the effect of grazing can be measured on an arithmetic scale of particle volume. With natural populations, however, it is also of interest to know the size fraction of particles being grazed as well as the quantity. For this purpose a logarithmetic distribution of particle diameter is best employed.

PROCEDURE

1. Obtain approximately 2-litre quantities of sea water containing phytoplankton and fill two 1-litre screw cap bottles with 900 ml of sea water each.*
2. Add from 2 to 20 copepods (ca 1 mg wet wt each) or equivalent biomass of zooplankton to one jar.
3. Place both jars on a slowly rotating wheel (ca one revolution/3 min) and incubate in the dark at constant temperature for a set time period.**
4. After incubation, remove the zooplankton by straining with a coarse net and measure the size spectrum of particles in each bottle as described in B. I (3). The total quantity of material grazed is obtained from the difference in biomass of particulate material between the samples with and without zooplankton. The size fraction grazed is given by the size fraction between the two points at which the spectrum of ungrazed particles joins or crosses the spectrum of grazed particles.

Example: The example shown in Figs. 22 and 23 is for Euphausia pacifica grazing on a mixed bloom of Chaetoceros socialis and Chaetoceros debilis during a period of 8 hours. From Fig. 23 it may be seen that the size fraction grazed is approximately between 12 and 80 μ diameter. From Fig. 22 the total quantity grazed (which can also be expressed as the quantity per animal) is $16.04 \times 10^6 \mu^3$ /ml. It is noticeable in Figs. 22 and 23 that in addition

* The exact amount of sea water, the number of zooplankton added and the size of the containers used is to some extent a matter of choice providing the volume of water per animal is greater than the quantity it could possibly filter during the incubation period. Since this will depend in part on the concentration of the phytoplankton in the water, the size of the container and the number of animals to add must sometimes be found experimentally. The volume of the container and the approximate number of zooplankters added as suggested here, have been taken from Mullin (1963) Limnol. Oceanogr. 8: 239-250 and have been found a satisfactory guide for our experiments.

** The temperature and time period of incubation are a matter of experimental choice. The in situ temperature of the seawater sample and a 24 hour incubation are suggested as a guide for initial experiments.

to the effect of grazing, there is also a measurable production of the smallest particles. This is believed to be a real effect resulting from the partial fragmentation of phytoplankton as they are grazed by zooplankton.

Fig. 22 Zooplankton Grazing
Location: Saanich Inlet
Date: 21/6/66
Incubation: 8 hrs dark

Diameter	No euphausiids	Euphausiids added
(μ)	Coulter Counter Volumes cu μ x 10^6/ml	
3.57	0.12	0.34
4.49	0.17	0.36
5.66	0.27	0.46
7.12	0.43	0.62
8.98	0.79	1.04
11.3	0.88	1.22
14.3	2.27	1.54
18.0	5.70	2.43
22.6	7.00	2.80
28.5	4.75	2.42
35.9	4.70	1.74
45.3	3.70	0.97
57.0	1.80	0.53
17.9	0.54	0.67
90.5	0.49	0.43
Total (cu μ 10^6/ml)	33.61	17.57
Quantity grazed (cu μ x 10^6/ml) 16.04		

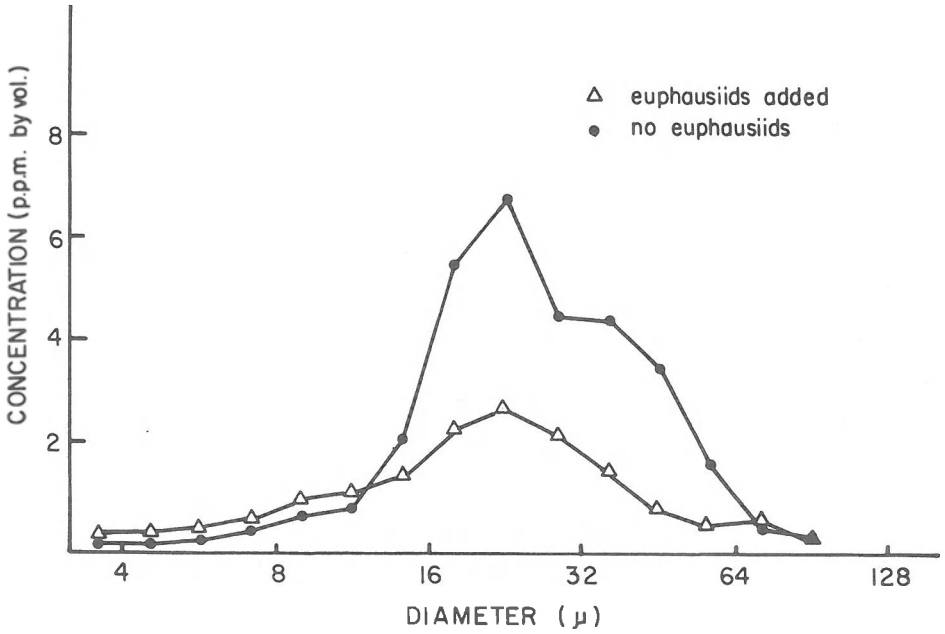


Fig. 23

IV. RECENT SEDIMENTS

(1) INTRODUCTION

Grain size analysis of sediments presents problems similar in some respects to those encountered with suspended particulate matter (see B. I). The range of particle size which can occur is enormous, varying from grains of clay mineral only a fraction of a micron in diameter to pebbles and cobbles whose diameter is measured in terms of centimetres. Only a small part of this range can be covered with the Coulter Counter. The normal working limits are from 1 μ to 500 μ. In ordinary investigations it is not usual to measure particles less than 1 μ, although this can be done if necessary. The range 500-1000 μ can also be measured with Coulter Counter but it is far easier to estimate grain sizes in this range by determination of settling velocity (e.g., Emery 1938). However, most marine sediments have grain size distributions in the range 1-500 μ.

A logarithmic scale of particle diameter should be used. The grade scale given in Fig. 5, is convenient. This is a 2 or $2^{\frac{1}{2}}$ scale of particle volume but based at 1 μ particle diameter. Since each diameter interval represents a doubling of particle volume, there are three grades between successive doublings of particle diameter. This scale, therefore, approximates to a 1/3 ϕ scale (Krumbein 1934). It is also very near to a $\log_{10}$ scale, and approximates to an Atterberg scale (every 10th interval), commonly used in soil analysis. This apparent similarity between a 2 scale and a 10 scale is simply due to the fact that $10^3 \simeq 2^{10}$.

Classical methods of sediment analysis measure the weight of sediment grains in each diameter interval or grade. Organic matter in the sediment has a density considerably less than that of mineral grains and for most sediments (with only a few percent organic material) it can be ignored. The Coulter Counter measures particle volume. A few percent of organic matter in a sediment may have negligible weight but it will have a significant volume. Before a grain size analysis is made the organic matter should be removed. With coarse sediments this can be removed by ignition. Fine sediments cannot be easily dispersed after ignition and organic material should be removed from these by wet oxidation. It is also desirable to keep fine-grained sediments wet during storage as some sediments can be difficult to disperse if allowed to dry out.

Methods of analysis depend on the range of grain size of the sample. Grains larger than about 100 μ cannot be maintained in uniform suspension in saline water, and it is necessary to increase the viscosity of the electrolyte. Glycerol can be added. Additions of 50-80% cause considerable increase in viscosity, but more than 80% cannot conveniently be used as the resistivity becomes too great. Suspensions should be made up in beakers with hemispheric bottoms, as these definitely help to achieve even dispersion, and samples should be stirred continuously.

Sediments can be conveniently divided into three groups: Medium sands, fine sands and silt, and mud. The analysis of each type is carried out differently and with an unknown sediment it is advisable to get an approximate idea of the grain size before beginning an analysis. This can be done simply by eye (for medium sands) by rubbing a small amount between the fingers (fine silty sands feel gritty, fine muds of similar bulk appearance feel smooth), or by washing a sample through one or two small sieves. Once an approximate idea of the range of grain size has been gained the appropriate method of analysis can be chosen. Selected methods are given below, but these may have to be modified in particular cases. The general principles of these methods should, however, always be applicable.

(2) MEDIUM SAND: size range 100-500 μ

Sediments of this type are widespread and form beach deposits and sand bars in locations where wave or current action is moderately vigorous. They are often very well sorted and may consist almost wholly of quartz grains. The size limits given have no physical reality but are just for the convenience of analysis. All intermediate types between fine, medium and coarse sands may be found. Coarse sands cannot be analysed easily with the Coulter Counter and fine sands should be analysed according to section B. IV (3).

PROCEDURE

1. Take a small sample (about 0.2 g) and place in a silica crucible.
2. If carbonates are to be removed add a small amount (usually about 5 ml) of 10% HCl.
3. Add distilled water and decant to remove the acid after the grains have settled.
4. Dry on hot plate at 110° C.
5. Ignite in furnace for 45 minutes at 550° C. At this temperature organic matter will be burned without affecting the mineral grains.
6. Allow to cool, and add a small amount of glycerol-saline. This should be made with 80% glycerol and 20% of 300 g/l NaCl solution.
7. Agitate in an ultrasonic bath for about 5 minutes to disperse the grains.
8. Make up to 250 ml with glycerol-saline. The easiest way to do this is to weigh a 300 ml round-bottom beaker. Wash the sample into the beaker with glycerol-saline, then make up to 300 gm (≡ 250 ml).
9. Using a 1000 μ aperture, check the particle concentration. If it is too great let the sample run away to waste via the aperture while maintaining a uniform suspension, and then refill the beaker. If it is necessary to know the amount of dilution the beaker can be weighed before and after adding glycerol-saline (see C.I(7)).
10. Count from 512-64 μ (Fig. 5), or between approximately these limits, using either the model M volume converter or automatic timer. With care, and a certain amount of experiment to determine optimum size for sub-samples, all necessary counts can be made on 250 ml sample.

Example: The distribution given in Fig. 24 is of sediment from a sand bar offshore from Comox Harbour, Vancouver Island. The sample was taken from about 10 metres.

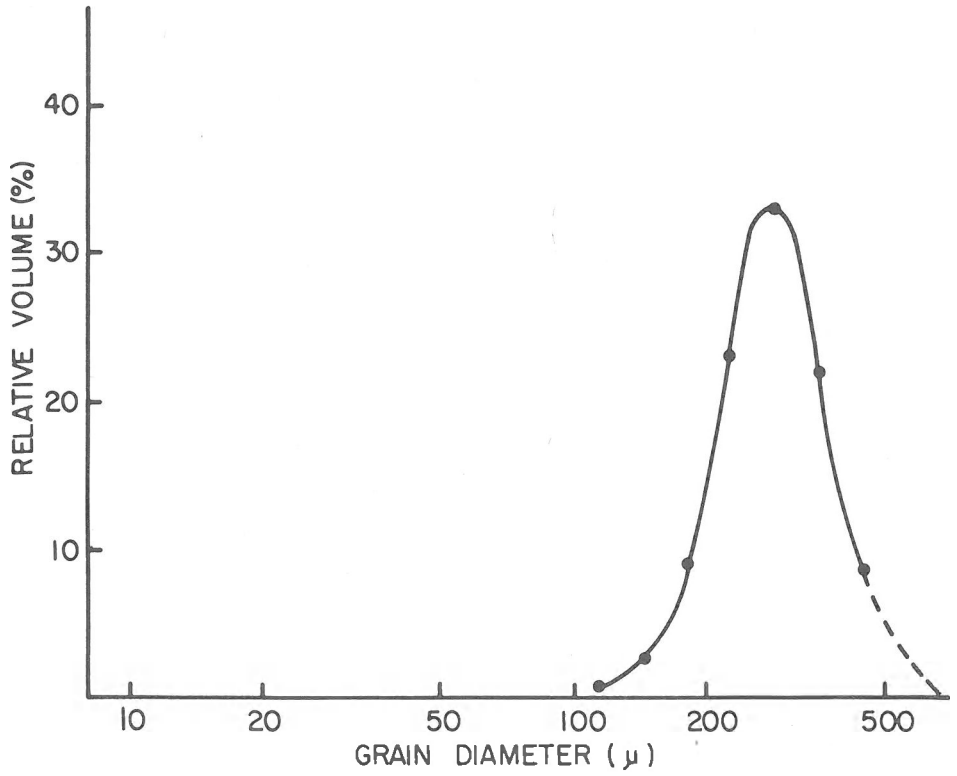


Fig. 24

(3) FINE SAND AND SILT: size range less than 250 μ

Whereas medium sands often contain little or no fine material, fine sands are often mixed with considerable quantities of silt. This is because fine sand accumulates in environments where wave or current action is only moderate, and because of this a certain amount of silt may also accumulate. It is usually possible to split the sample at about 25 - 40 μ and analyse each fraction separately.

PROCEDURE

1. Take a small sample (about 0.1 gm) and wash into 50 ml beaker with a minimum of distilled water.
2. Add 2 ml of 30% H_2O_2.
3. Put the beaker on a hotplate so that the temperature of the sample is about 70° - 80°C, and leave to simmer overnight. The hydrogen peroxide will decompose and digest readily oxidizable organic matter.

4. If carbonates are to be removed add a small amount of acid. The amount and type of acid used will depend on the type of sediment analysed. For instance, a sediment with only a little calcite and much clay mineral would need a small amount of weak acid (e.g., acetic), but a sediment with a considerable amount of dolomite and no clay mineral could be treated with a strong acid (e.g., hydrochloric).
5. Put the sample in an ultrasonic bath and agitate for about 5 minutes. Add a little saline if necessary. The saline should be at the concentration for which the counter is calibrated, preferably 1% NaCl. However, if the instrument is also used for counting particles in seawater 3% NaCl should be used.
6. Wash the sample on to a sieve (25 μ or 37 μ mesh spacing) with saline. The material passing the sieve should be collected into a 250 ml volumetric flask.
7. Wash the material retained on the sieve into a crucible and dry at 110°C.
8. Ignite at 550°C. This is necessary to remove relatively large particles of organic matter not completely digested by H_2O_2.
9. Allow to cool and add a small amount of glycerol-saline. This should be made with 50% glycerol, 10% 300 g/l NaCl, and 40% distilled water.
10. Follow procedures 7 to 10 in B. IV (2), but make up to 287 g (≡ 250 ml) with glycerol-saline and use a 560 μ aperture. Dilutions can be made either by letting some of the sample run to waste, or by sub-sampling with a pipette and then making up to 287 gm in a beaker, (C.I(7)). Counts should be made from 256 to 20 μ.
11. Check the concentration of the suspension which passed the screen (i.e., that obtained from Procedure 6) using a 100 or 140 μ aperture and dilute if necessary (for pipette and volumetric flask see C.I(7)).
12. Take counts over the range from 40-50 μ to 3-4 μ.
13. If necessary a sub-sample can be taken, washed through a 25 μ screen and counts in the range 10-1 μ made using a 50 μ aperture.

Since each fraction of the sediment was originally made up to 250 ml the relative proportions of each grade can be calculated, provided that all dilutions are recorded. Example: Figure 25 is a fine sand with an admixture of silt from the Strait of Georgia, British Columbia. This is a very common type of fine sediment. The sample was divided at 37 μ (A.S.T.M. standard screen) and each fraction was made up to 250 ml. The fine material was diluted 10 times and the coarse material 2.62 times. The results for each fraction were calculated in terms of concentration (ppm by vol.) of particles in the original 250 ml samples and are therefore comparable. The absolute values of concentration are, of course, unimportant as these depend on the size of the original sediment sample. The results could be recalculated in terms of percent of total sample.

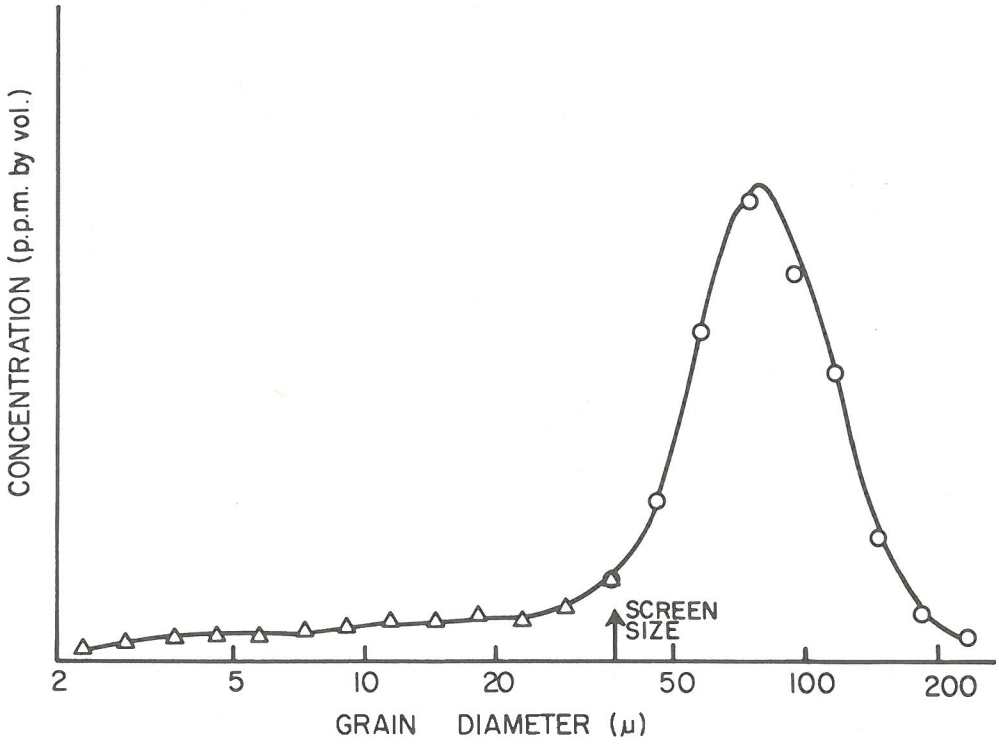


Fig. 25

(4) MUD: size range less than 100 μ

This is probably the easiest sediment to analyse and is also the least common in occurrence. It is found in areas where wave action is absent and currents are weak. Sediments of this type probably accumulate mainly from material carried in suspension.

PROCEDURE

1. Prepare a sample as described in B. IV (3), Procedures 1 to 5.
2. Make up to 250 ml with NaCl solution (see B. IV (3), Procedure 5), then analyse following Procedures 11 and 12 of B. IV (3), but use a 200 or 280 μ aperture and count over the range 100 μ to 5 μ.
3. If necessary a sub-sample can be taken and counts made from 10 μ to 1 μ as described in B. IV (3), Procedure 13.

Example: Fig. 26 shows the grain size distribution of a mud. The sediment was taken at a depth of 100 m from the Strait of Georgia, British Columbia. The analysis was made with a 200 μ aperture only.

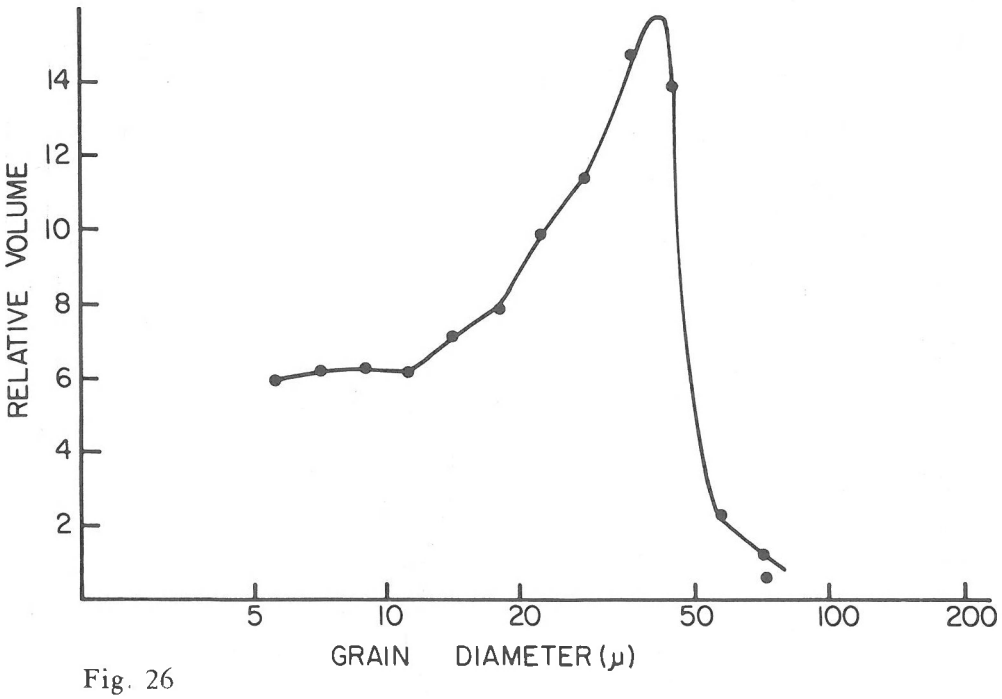


Fig. 26

V. SOME OTHER APPLICATIONS

(1) DENSITY MEASUREMENT

Since the Coulter Counter measures particle volume, the total volume of particles in a sample can be calculated simply by multiplying the number of particles and the mean particle volume for each grade. If the total weight of a sample is also known the average density of the particles can be determined. Open-ended distributions cannot be measured, but screening between known upper and lower limits of particle size will effectively close an otherwise open distribution. For instance, the sample shown in Fig. 25 forms a suitable distribution if only the material retained by a 37 μ screen is considered.

It is often possible to make density measurements as part of other determinations and it is rarely necessary to analyse samples for density alone. In the examples given in Fig. 27, (a) was obtained from a series of analyses of a medium quartz sand. The basic purpose of the analyses was to obtain information on particle-size distribution. However, the samples were weighed after Procedure (5) (B. IV (2)) and it was then possible to plot weight against

volume and so obtain the mean density of the particles. The value obtained (2.6 gm/ml) is very close to the density of quartz (2.66 g/ml). This serves as a useful check on the accuracy of volume determinations. Similarly, example (b) is for a series of determinations of grain size distribution of a silty sand. The fraction retained by a 37 μ sieve was weighed after Procedure (8) (B. IV (3)). The average density of the particles (2.0 g/ml) is less than that of quartz. This was because many of the grains were sponge spicules. These have a density less than quartz and are also hollow.

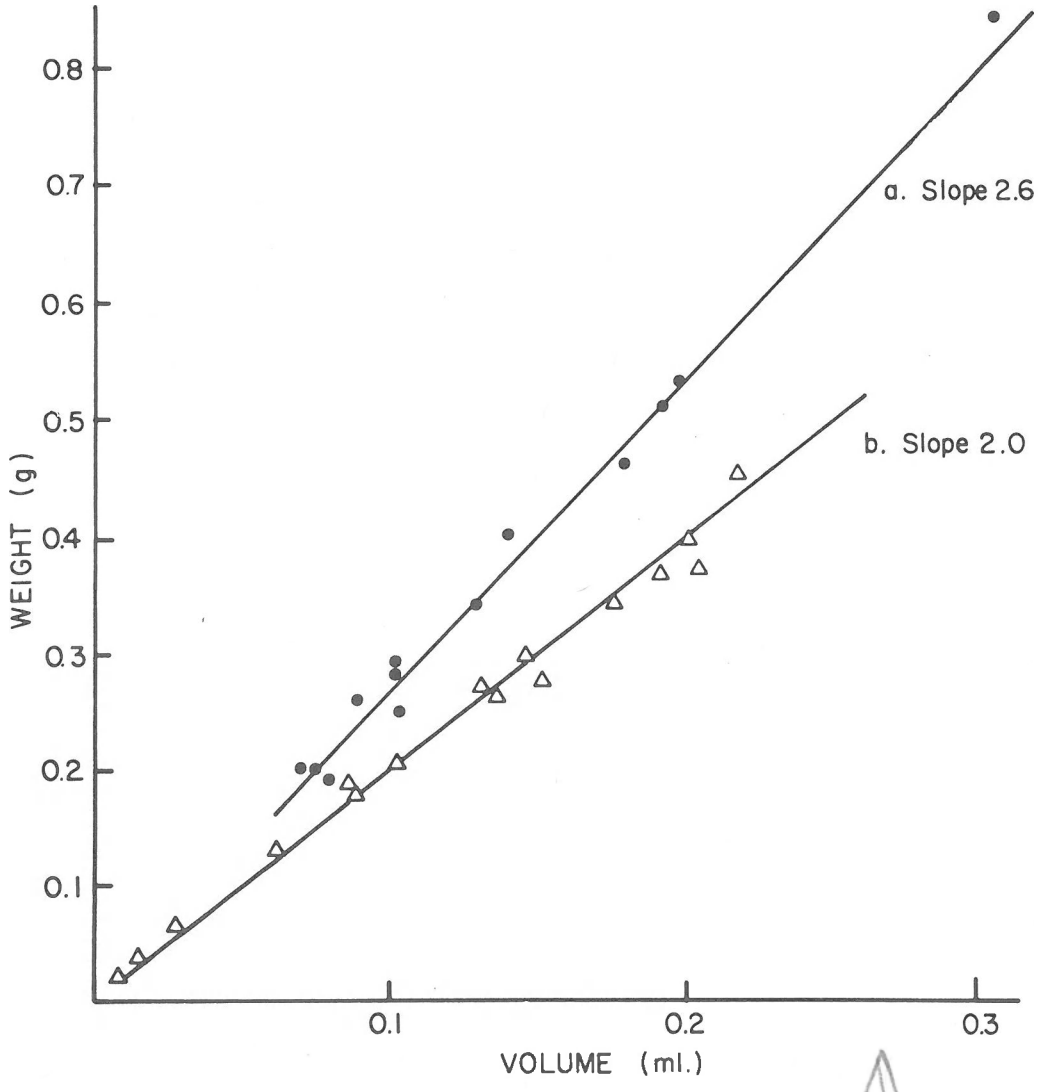


Fig. 27

useful exercise for natural particles

(2) ANIMAL MIGRATIONS

One feature of the Coulter Counter of considerable potential value is that it is possible to accurately measure grain size distributions of very small samples. The minimum sample size depends to some extent on the grain size, but distributions of fine-sand samples of .02 g can be measured accurately.

Many bottom dwelling animals carry small amounts of sand in their stomachs (Sheldon and Warren 1966). This is either picked up accidently while feeding or is actually in the food (e.g., in the gut of worms). If the pattern of grain size distribution of the bottom sediments is known in detail it is sometimes possible to find where feeding occurred and whether migration from the feeding area has taken place. This is done simply by comparing the grain size distributions of the bottom deposits with that of the small amount of sand in the stomach. If the two distributions differ then the animal must have moved since it last fed. The movement of a skate (Raia batis) over more than 20 km has been detected in this way (Sheldon and Warren 1966) and the feeding ground of a shrimp (Pandalus montagui) population has been discovered (Warren and Sheldon 1967).

(3) GRAIN SIZE DISTRIBUTIONS OF VARVED SEDIMENTS

It is possible, by taking very small samples, to measure the grain size distributions of individual layers in varved sediments.

Figure 28 shows particle-size distributions for six samples of sediment taken at about 1.3 mm intervals from the upper part of a short core from Saanich Inlet, British Columbia. Each had a volume of less than 10 cu mm (i.e., the volume of the sample as taken from the core not the total volume of particles). Sediments from Saanich Inlet have been described by Gross et al (1963) and Gucluer and Gross (1964). They are banded with light and dark layers and it is believed that the banding is annual, with one light and one dark layer formed each year (i.e., varved).

Analyses were made as described in B. IV (4) and were made with a 50 μ aperture.

Two types of distribution can be recognized with modes at about 2.0 μ and about 4.0 μ. The sampling interval (about 2.6 mm for two layers) was similar to the estimate for annual deposition (2 mm) given by Gross et al (1963), and it is possible that the distributions represent sediment deposited at different times of the year.

(4) CARBON CONTENT AND PARTICLE VOLUME

The difficulty of deciding exactly what the counter measures in terms of biomass has been one of the more controversial points to resolve when working with this type of instrument. For most practical purposes the response of the instrument to irregularly shaped particles appears to be essentially proportional to particle volume. This is illustrated in Fig. 29 where more than 70 measurements of total particle volume have been correlated ($r = 0.88$) with particulate

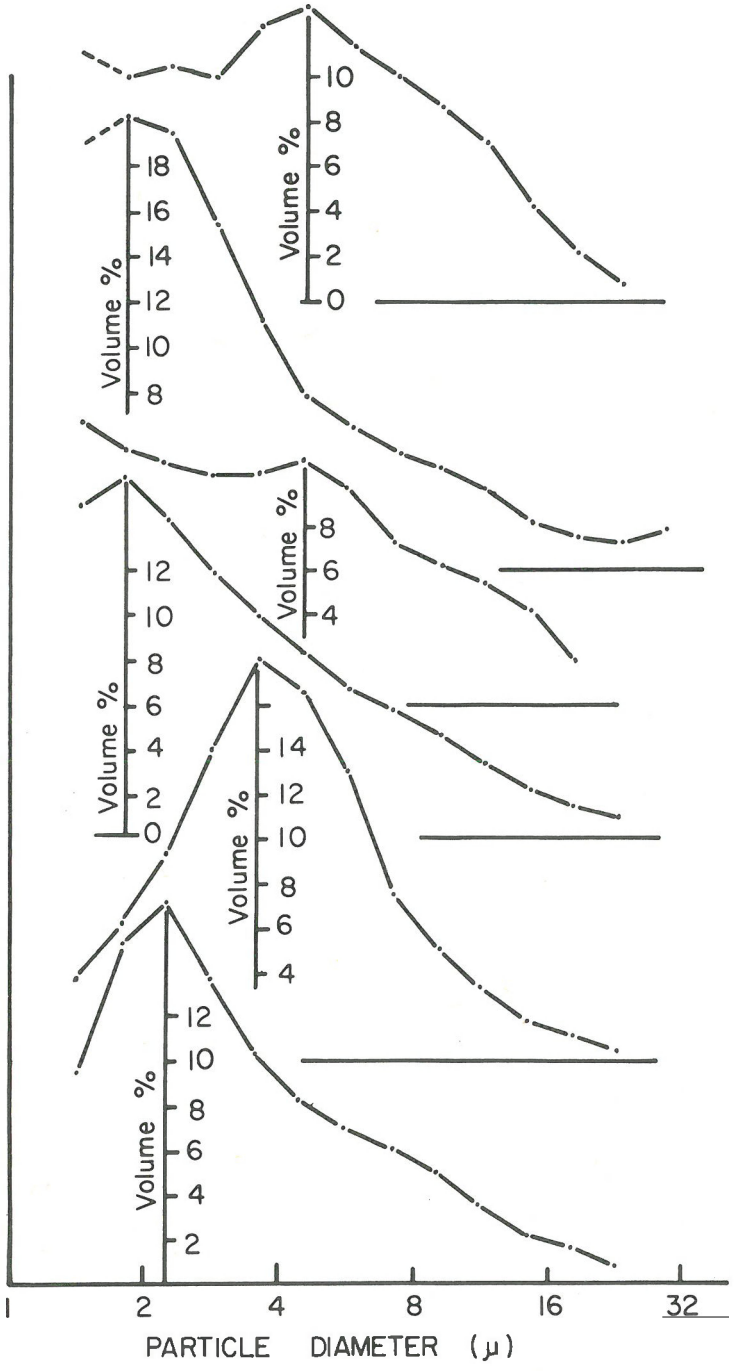

Volume %
10
8
6
4
2
0
Volume %
18
16
14
12
10
8
Volume %
8
6
4
Volume %
12
10
8
6
4
2
0
Volume %
14
12
10
8
6
4
Volume %
12
10
8
6
4
2
1
2
4
8
16
32
PARTICLE DIAMETER (μ)

Fig. 28

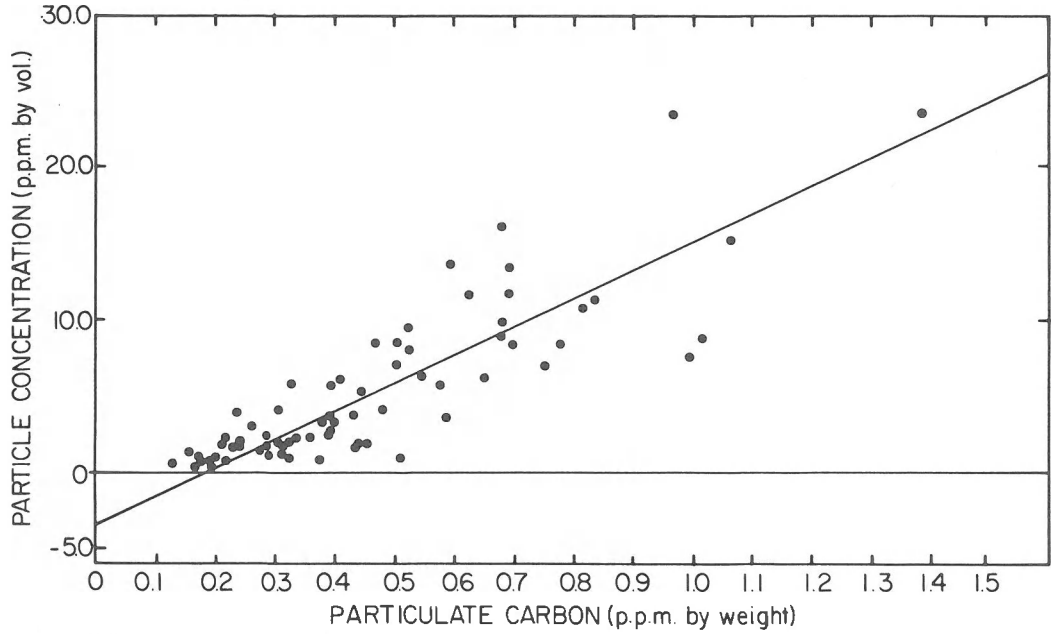


Fig. 29

carbon (Sheldon and Parsons 1967). This correlation was obtained in spite of the fact that many chain-forming diatoms were present among the particulate matter.

For greater particle size ranges the correlation of carbon in plankton populations with Coulter Counter volume determinations may be logarithmic since larger plankton species contain relatively smaller proportions of carbon (Mullin et al 1966). For any environment, however, if some absolute measure of biomass is required from volumes determined with the Coulter Counter, this would be best obtained from a predetermined regression line such as is shown in Fig. 29.

SECTION C.

APPENDICES

I. NOTES

(1) DISPERSION OF POLLEN GRAINS

Calibration depends on the measurement of mono-sized particles. It is essential, therefore, that they are dispersed as single particles. If large numbers are sticking together in twos and threes the calibration will be worthless.

To disperse: Put about 1/4 cc pollen into a 50 ml sample tube. Half fill it with seawater and add 1 drop of detergent. Shake vigorously and then allow it to stand. Repeat the shaking at 15 minute intervals for 1 hour and then leave for 24 hours. A few drops of this stock suspension should be poured through a screen into 250 ml of seawater. This will give approximately the right concentration of particles. The screen should be larger than a single grain but smaller than a doublet (e.g., for ragweed pollen, a 25 μ screen will allow very few multiple particles to pass). The stock suspension can be kept for a further two days, but should then be discarded.

(2) PREPARATION OF PARTICLE-FREE SEA WATER

All natural sea water contains particles, but sea water used for making up and diluting particle suspensions must be particle-free. Filtration is the most convenient method for particle removal and the following filter-types are recommended:

For 560 μ and larger apertures ----- glass-fibre filter pads, pore size 3-5 μ

For 100-400 μ apertures ---------- membrane filter, pore size 0.5-2.0 μ

For 70 μ and smaller apertures ----- membrane filter, pore size less than 0.5 μ

It is also possible to remove particles with a continuous-flow centrifuge. Flow rate and speed of rotation can be adjusted so that particles are removed to any predetermined size. Actual values are found by experiment and will depend on the type of instrument used.

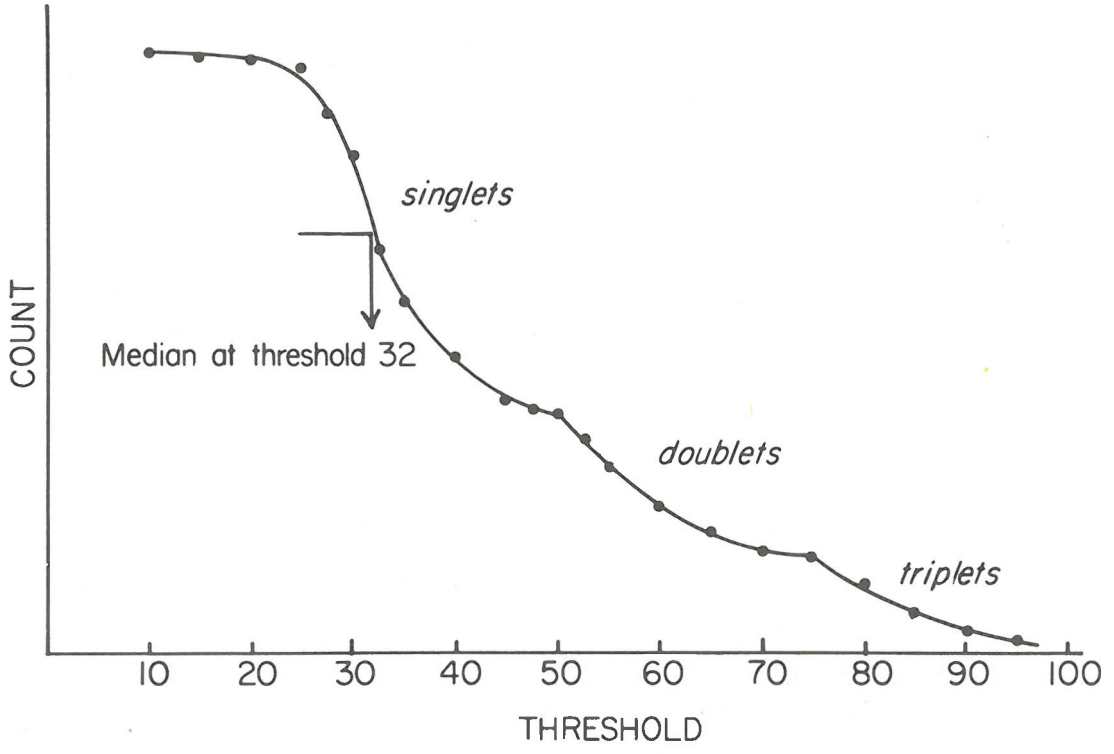
Organic particles can form in membrane-filtered sea water (Sheldon, Evelyn and Parsons 1967) so that it may remain particle-free for only a few hours. It is always better to use freshly filtered sea water.

(3) CALIBRATION OF SMALL APERTURES

It is relatively easy to completely disperse large particles (e.g., ragweed pollen), but suspensions of small particles (i.e., less than about 10 μ diameter) may often contain numerous doublets and triplets which are difficult to separate. The median of a series of cumulative counts will not be a measure of average particle size. It is necessary to divide the curve into parts, each representing a different kind of particle, and then calculate the median for the population of single particles (Fig. 30).

In most cases it is not difficult to recognize each part of the cumulative curve, but it is often better to record counts in the form of a differential frequency distribution. To do

this put the upper threshold mode switch to LOCKED. The upper threshold will now produce a threshold "window" 1/10 the number of divisions of the upper threshold setting. For example, set the upper threshold at 50. This will give a threshold "window" of 5 divisions, and the lower threshold control will move the "window"; i.e., with the lower threshold set at 15, counts will be between threshold settings 15 and 20; set at 16 between 16 and 21, etc.



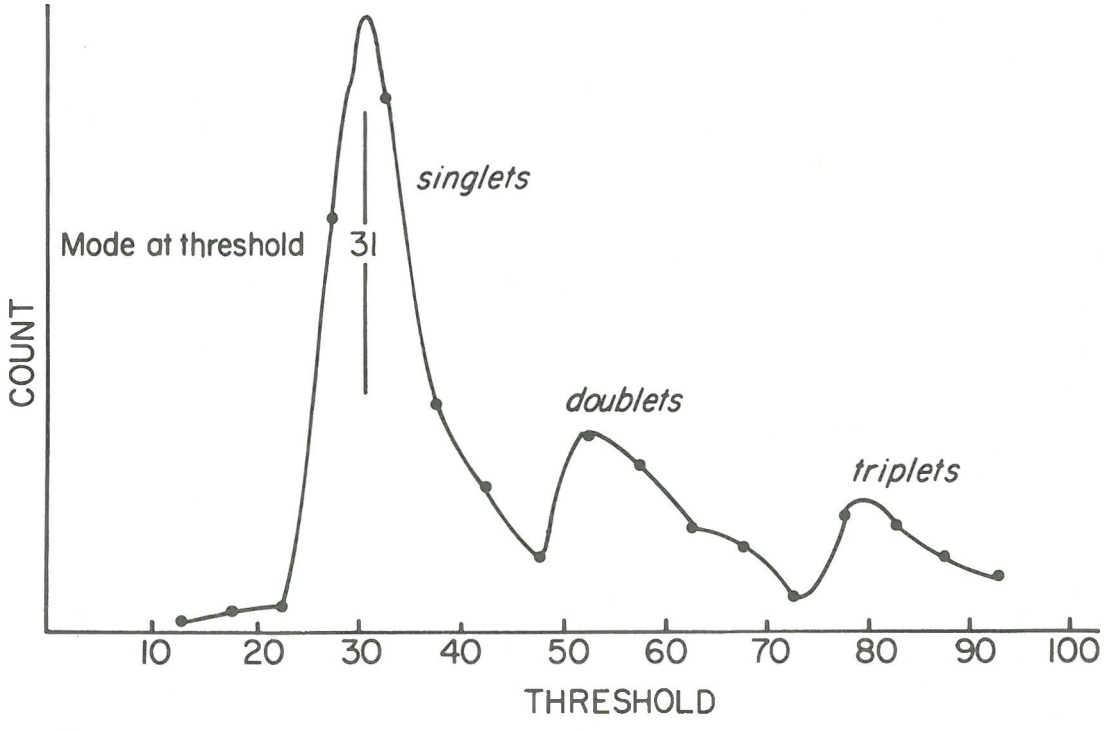


Fig. 30

Take counts at convenient points and plot a differential frequency distribution (Fig. 30). The mode of the peak representing single particles will give a measure of particle size. The mode can be found by measuring half-way between the flanks of the peak (Fig. 30).

(4) SCREENING

Particle distributions with a large range of size cannot be measured with a single aperture, and at some point in the analysis the sample has to be screened to prevent blocking of a relatively small aperture by large particles. The efficiency of the screening depends on the nature and shape of the particles and on the method of using the screen.

If the particles are sub-spherical and can be washed into the screen then it will separate particles almost exactly as the mesh-size (see B. IV (3)).

If the material cannot be washed on to the screen but must simply be poured through (e.g., seawater samples, where dilution of the sample passing the screen would be unacceptable) then many particles smaller than the mesh will stick to the wires and to other (large) particles.

If, in addition, the particles are not approximately spherical the screen will retain particles with an effective diameter (based on volume) considerably less than the mesh spacing. For example, consider a mixture of spherical beads (diameter 7.5 mm) and nickels (5¢ coins). A sieve with a mesh of a little less than 10 mm will pass all the beads and retain all the nickels, yet the volume of a nickel is such that its effective diameter is 6 mm (i.e., diameter of sphere of same volume as the nickel). Particles of various shapes occur in the sea and many are even less substantial than a disc, so that in practice a screen will retain particles from seawater far smaller than the mesh spacing would suggest.

In general the following approximate limits should be observed:

(a) Sub-spherical particles washed through a screen: separated to about 20% of the mesh spacing, i.e., if the material is screened at 50 μ then the distribution will not be affected at diameters less than 40 μ.

(b) Irregular shaped particles washed through a screen: the limits here will depend very much on particle shape but a good general rule would be to allow for effects to 50% of the mesh spacing, i.e., if the material is screened at 50 μ then the distribution will not be affected at diameters less than 25 μ.

(c) Irregular particles poured through a screen: here the effect of the screen is considerable. With suspended matter in seawater the effect can extend to 80% of the mesh spacing, i.e., if the material is screened at 50 μ then only below 10 μ will the distribution be unaffected.

When making measurements over a wide range of particle size there are various combinations of apertures and screens which can be selected. In choice of screen size a compromise often has to be reached between a screen which will not affect the particle-size

distribution within the range of a particular aperture, but will probably pass particles large enough to block the aperture, and one which will filter off all particles capable of causing blockage, but as a consequence of this may affect the size distribution well into the working range of the aperture. In any particular series of observations it is best to determine by experiment the most suitable combinations of screens and apertures to use. Also, in some cases, considerable overlap in the ranges of the apertures used may be necessary.

There is a similar, but less pronounced, effect on particles retained by a screen. We have seen that particles with effective diameters smaller than the mesh spacing can be retained, but if the particles are cylindrical or ellipsoidal some with effective diameters greater than the mesh spacing may be passed. This effect can extend to diameters 10% greater than the mesh spacing.

(5) CALCULATION TO DETERMINE THE AMOUNT OF DETRITUS IN SUSPENDED MATTER IN THE SEA

The theory behind the procedure followed is given by Cushing and Nicholson (1966). In brief, the expression

$$\frac{V-\hat{D}}{V_o-\hat{D}} = e^{\hat{k}(t-t_o)}$$ is used to obtain an estimate of k, the true growth constant. V_o is the total quantity of particulate material at the beginning of the incubation (time, t=O), V is the total quantity of particulate material at time t, $\hat{D}$ is an estimate of the true volume of detritus (D) and $\hat{k}$ is the corresponding value of k. Since the true growth constant (k) will not change with time, values of $\hat{k}$ are plotted against time, and the value of $\hat{D}$ at which $\hat{k}$ vs t has zero slope is taken as a measure of the true volume of detritus (D). By substituting this value of D back into the above equation, the growth constant of the phytoplankton can be obtained.

(6) EVALUATION OF METHODS FOR DETERMINING THE GROWTH RATE OF NATURAL PHYTOPLANKTON POPULATIONS

The assumption is made in the calculations (B. II (3) (b)) that D, the volume of detritus, remains constant during the 72 hour incubation. This may not be correct if dissolution, precipitation or bacterial growth occurs. The length of the incubation period and the assumption regarding the detritus are disadvantages of this method. The other method (B. II (3)(a)), in which no correction is made for detritus, gives a measure of growth rate which can only be employed in calculations involving short time intervals unless the population of phytoplankton greatly exceeds the quantity of detritus. Such conditions may only occur in isolated environments or during heavy blooms. Thus, in summary, either of two methods, or a combination of both, may be employed depending on the type of information required.

(7) DILUTION METHODS

The easiest way to dilute is to take a subsample with a pipette and then make this up to a known volume in a volumetric flask. This method can be used with suspensions of particles with low settling velocities (i.e., either small size or low density). Large, high density particles (e.g., sediment grains larger than about 50 μ) tend to settle out in a volumetric flask so that the sample cannot be transferred quantitatively from the flask to a beaker for measurement. Dilutions should be made in beakers. There are two convenient ways to do this:

(a) By taking the concentrated suspension away to waste through the aperture and then making up the remaining suspension with particle-free electrolyte. The dilution can be calculated by weighing before and after adding the electrolyte.

(b) By taking a subsample with a pipette, washing this into a beaker and then making the sample to some predetermined weight.

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