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A simple weighing and dilution technique for determining absolute abundances of coccoliths from sediment samples

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Abstract An easy to use, quantitative technique is described for preparing nannofossils from sediment samples for examination in light-microscopes. Crucially, this method keeps fractionation and dissolution of small coccoliths to an absolute minimum and uses few consumables or expensive equipment. Comparisons between independently made samples show limited statistical difference between nannofossil abundances.

Keywords Quantitative preparation technique, calcareous nannofossils

1. Introduction

Coccolithophore abundance counts per gram of sediment are important for determining nannofossil accumulation rates and have potential value in the development of proxies for sea-surface temperatures and nutrient levels. However, such measurements are prone to irregularities caused by the very methods that are used to prepare the nannofossils for study. Problems with unequal settling, unevenness of cover, ease of preparation and study have prompted authors to publish many different methods for quantifying nannofossil abundances, including methods based on random settling, filtration or spiking with microbeads (Bown & Young, 1998).

The method described in this paper has been developed for routine application during palaeoceanographic research, and applied to several cores of Late Quaternary muds and calcareous oozes from the NE Atlantic. The high abundances of small coccoliths from the Family Noelaerhabdaceae, such as *Emiliania huxleyi*, *Gephyrocapsa oceanica* and *G. ericsonii*, require a preparation technique that will cause minimal selectivity between size-fractions. Almost equally important, is to minimise the possibility of alteration to the coccoliths. Any dissolution of smaller members of the Noelaerhabdaceae has the potential to seriously distort species counts (Gibbs *et al*., 2004), and to make identification of small species very difficult.

Random settling techniques (Beaufort, 1991; Okada, 1992; Flores & Sierro, 1997) have been successfully applied to many samples of the type described above. However, they always had the possibility of altering the finer fractions, no matter how carefully the water/sediment mixture was mixed, or how carefully the water was siphoned off. The prolonged immersion in water that these methods require is also undesirable, given the possibility of altering the coccolith preservation.

Preparing samples using filtration techniques (McIntyre & Bé, 1967; Andruleit, 1996; Herrle & Bollman, 2004) has also been used successfully in the past, especially for quantitative SEM studies. Unfortunately, filters are less useful under the light-microscope, where their undesirable characteristics present problems. Polycarbonate filters are very unsatisfactory for light-microscopy, since the membrane is not completely transparent, the pores remain highly visible, and the membrane acts as a single crystal unit under cross-polarised light. Cellulose nitrate and cellulose acetate filters have much better optical properties; they are nearly transparent in mounting media and are non-birefringent. However, they still do cause slight image degradation, especially in phase-contrast and at focal depths within 1-2μm of the filter surface. Also, they have uneven surfaces and, in consequence, a substantial proportion of specimens are tilted, which reduces the reliability of morphometric measurements. For preparation of large numbers of samples, the time required for filtration, and the cost of these filters, can also be an issue. Other methods, the spiking with microbeads and spraying method (Bollman *et al*., 1999) for instance, require expensive equipment, such as precision microbalances and microbeads.

What is needed, ideally, is a method that avoids any size-separation effects, but that also can be prepared on a glass slide for the best possible optics with minimal equipment. The methods discussed above have adopted relatively elaborate preparation procedures in order to produce homogeneous particle distributions. This seemed a relatively labour-intensive solution to the problem, so we undertook some experimentation, with variations on pipetting techniques and an analysis of the problems. We found that the main cause of uneven distribution was the movement of suspension during drying, resulting, in extreme circumstances, in multiple concentric drying-rings on the slide. This problem, however, can be minimised by rapid drying on a flat hot-plate and by taking care to count specimens from fields of view distributed across the slide.

Following from this, we developed a modified version of the classic pipette strew technique, as described for instance by Bown & Young (1998), with incorporation of steps to allow reliable quantitative nannofossil data.

2. Procedure

This method basically involves pipetting a known quantity of sample onto a known surface area. It is easily scaled up and is more efficient if many samples are prepared at the same time. Once proficient in the method, it typically takes 45 minutes to complete a batch of 24 samples. The only consumables are the slides themselves. Test-tubes, pipette-tips and test-tube caps can be cleaned and reused, if needed. Entering data into a spreadsheet while working helps keep track of the data and perform the simple calculations required for later stages, an example of this is shown in Figure 1. Each step is carried out on all samples in the batch before proceeding to the next step.

1. Oven dry, or better freeze dry, the sediment. *NB* This is necessary to allow accurate weighing of the sample and greatly aids the disaggregation of clay-rich samples.

2. Remove a subsample of about 0.1-0.2g of dry sediment; weigh using a microbalance with precision of 0.001g, and transfer into a 10ml test-tube. Using a micropipettor, add 10ml of buffered, distilled water and disperse by shaking. *NB* Achieving complete disaggregation of the sample at this stage is essential for the results to be meaningful. Some samples require more severe treatment than simple shaking, such as ultrasonication or use of dilute hydrogen peroxide (for organic-rich sediments), but beware of damaging the nannofossils. For buffering, we use a few drops per litre of ammonia and test the water using a pH strip.

3. Calculate the volume of suspension which contains 0.008g of sediment and after mixing thoroughly, use a micropipettor to transfer this volume to a second test-tube (0.08/weight of dry mud x 1000 = millilitres of suspension into second test-tube). Fill this test-tube up to 10ml with buffered, distilled water. *NB* The value of 0.008g was found to be ideal for clay-rich sediments; for calcareous oozes, a lower volume (about 0.002g) was used. Dilute suspensions are more vulnerable to dissolution, so it is important to avoid delay before proceeding to Step 4.

4. Pipette 100μl of the well-mixed second solution onto a coverslip, ensuring it covers the entire surface. We use 32x22mm coverslips. Dry quickly on a flat hot-plate. *NB* The heat setting of the hot-plate needs to be adjusted to promote rapid drying without boiling, as sediment can be lost by spitting.

5. Mount the coverslip onto a slide using your preferred method; we use Norland Optical Adhesive.

	Test tube weight / g	Test tube + mud / g	Mud weight / g	ml to 2nd test tube for 0.008g	weight of mud on coverslip / g	Coverslip area / mm^2	Area one FOV / mm^2	Number of FOVs counted	Number of specimens counted	Number of total specimens per gram of sediment
OMEX 2K 107cm	4.7538	4.8987	0.1449	552	0.00008	704	0.045	50	740	2,894,222,222

Figure 1: Summary spreadsheet table used during this method, with the example of OMEX 2K, 107cm. Entering the data while working helps with the simple calculations required in Step 3

Since the weight of sediment transferred to the slide is known, counts of coccoliths per field of view can readily be converted into numbers of specimens per gram of sediment and, potentially, into flux rates. The number of specimens can be calculated as follows:

Number of specimens/gram of sediment =

$$\frac{A * N}{f*n*W},$$

where A = coverslip area; f = area of one field of view; n = number of fields of view counted; W = weight of sediment on coverslip; and N = number of specimens counted.

3. Reproducibility and accuracy

This method was developed and applied during the study of two cores from the same location in the Porcupine Bight area of the NE Atlantic Ocean, OMEX 2K and MD04 2819 (49˚5'N, 13˚26'W), both containing high-resolution records of the Late Quaternary. Nannofossil abundances vary considerably in these cores, from interglacial foram-nannofossil oozes, to near barren samples from Heinrich Layers ($3.5x10^{11}$ to $2.6x10^{7}$ nannofossils per gram of sediment). Samples are dominated by small Noelaerhabdaceae (*Emiliania huxleyi* and various members of the genus *Gephyrocapsa*).

Eighteen samples from OMEX 2K were prepared twice (the entire method was repeated, including fresh sediment), and full counts of each slide performed; 50 randomly selected fields of view were counted and at least 200 coccoliths per sample counted. Samples at 77, 83 and 89cm had extremely low abundances (related to Heinrich Event 1), so as many coccoliths were counted as practically possible on these slides. One-hundred-and-ten single samples from MD04 2819 were prepared and counted in the manner described above and successfully used in the study of this core (Koch, unpub. data). Abundance comparisons between the pairs of samples from OMEX 2K show a correlation coefficient of 0.9432 (Figure 2).

4. Conclusions

This method ensures that little or no fractionation occurs, which is critical when analysing samples with high abundances of small placoliths. It scales up well, so processing many samples is very easy and, since this method does not require settling in a stable environment, it is suitable for preparation aboard ships. The reproducibility results show that the method provides minimal margin of error when calculating absolute abundances of calcareous nannofossils.

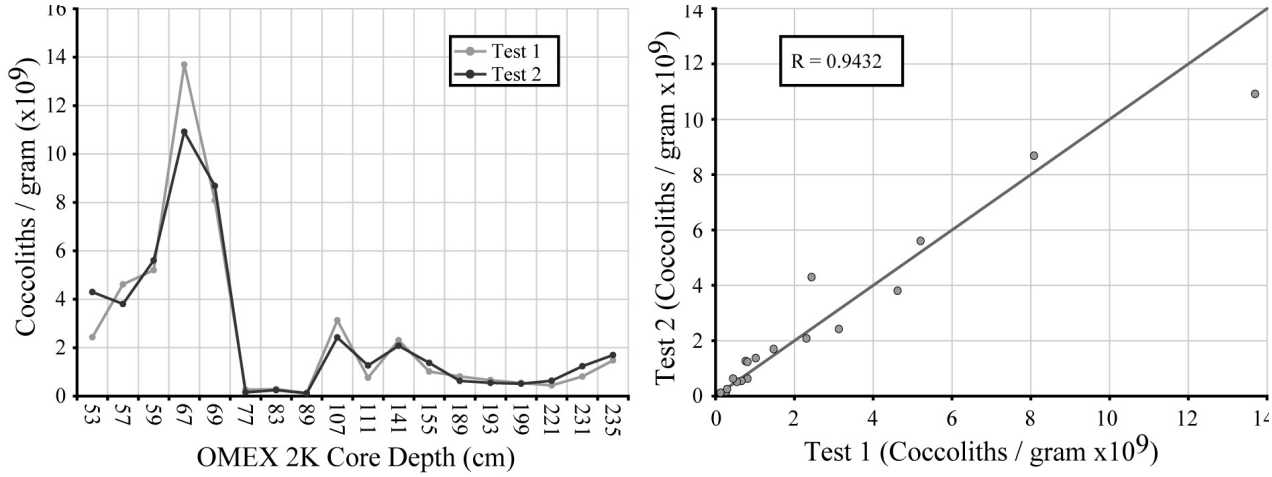

Figure 2: Abundance comparisons for the two rounds of sample preparation, from 18 OMEX 2K samples. **Left-hand graph**: Down-core variation in coccolith abundance, with test one and test two count series plotted separately. **Right-hand graph**: Cross-plot of counts from the two series. They show minimal scatter in values across the abundance range of the samples (R=0.9432)

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5. References

Andruleit, H. 1996. A Filtration Technique for Quantitative Studies of Coccoliths. *Micropaleontology*, **42**(4): 403-406.

Beaufort, L. 1991. Adaptation of the Random Settling Method for Quantitative Studies of Calcareous Nannofossils. *Micropaleontology*, **37**(4): 415-418.

Bollman, J., Brabec, B., Cortes, M. & Geisen, M. 1999. Determination of absolute coccolith abundances in deep-sea sediments by spiking with microbeads and spraying (SMS method). *Mar. Micropaleontol*., **38**: 29-38.

Bown, P.R. & Young, J.R. 1998. Techniques. *In:* P.R. Bown (Ed.). *Calcareous Nannofossil Biostratigraphy*. British Micropalaeontological Society Publications Series. Chapman & Hall/Kluwer Academic Press, London: 16-28.

Flores, J.A. & Sierro, F.J. 1997. Revised technique for calculation of calcareous nannofossil accumulation rates. *Micropaleontology*, **43**(3): 321-324.

Gibbs, S.J., Shackleton, N.J. & Young, J.R. 2004. Identification of dissolution patterns in nannofossil assemblages: A high-resolution comparison of synchronous records from Ceara Rise, ODP Leg 154. *Paleoceanography*, **19**: PA1029.

Herrle, J.O. & Bollmann, J. 2004. Accuracy and reproducibility of absolute nannoplankton abundances using the filtration technique in combination with a rotary sample splitter. *Mar. Micropaleontol*., **53**: 389-404.

McIntyre, A. & Bé, A.W.H. 1967. Modern coccolithophoridae of the Atlantic Ocean: I. Placoliths and cyrtoliths. *Deep-Sea Research*, **14**: 561–597.

Okada, H. 1992. Use of microbeads to estimate the absolute abundance of nannofossils. *J. Nannoplankton Res*., **14**: 96-97.