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Smear and spray preparation techniques put to the test (II): Reproducibility and accuracy of calcareous nannofossil assemblage counts

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Abstract In an earlier study (Henderiks & Törner, 2006, *Marine Micropaleontology*, **58**: 207-218), nannofossil proportion estimates between replicate smear-slides showed substantially higher variance than those obtained from replicate sprayed slides. This study revisits this potentially disconcerting issue, detailing the internal accuracy and reproducibility of relative nannofossil species abundances obtained with the same two preparation techniques: the simple smear-slide and the spray method. In addition, accuracy and reproducibility were tested for a semi-quantitative method, in which the number of specimens per mm^2 is determined from smear-slides.

To test the overall reproducibility of these two preparation methods, replicate slides were prepared and analysed with each technique for a set of six samples selected from Oligocene deep-sea sediments from the equatorial Pacific (ODP Leg 199). Both smear and spray replicates indicate a good reproducibility of proportion estimates within the same method, since no statistically significant differences between the replicate slides were observed. However, when comparing the smear with spray replicates, the dataset reveals significantly different species proportions between slides, indicating that the two preparation techniques are not statistically comparable. It appears that the dominant taxon, *Cyclicargolithus floridanus*, is enriched in the smear-slides, whereas the abundance of the subordinate taxa is about the same for either method. A comparative test of abundance counts between dense and thin areas on the same smear-slide indicates nannofossil size sorting in the smearing technique, with proportionally more large specimens in the dense ripples. This study confirms an earlier observation, that the smear method results in lower proportion estimates of larger nannofossil taxa in comparison to the spray method.

Keywords calcareous nannofossils, preparation techniques, light microscopy, accuracy, reproducibility, biostratigraphy

1. Introduction

Collection of biostratigraphic and palaeoecological calcareous nannofossil data requires reproducibility tests of different techniques, to ensure the gathering of good quality records. There are important questions concerning data accuracy, consistency and reliability that must be considered in data interpretation, as well as comparison between different datasets. In recent years, increasing interest is being shown in the comparability of datasets obtained using different sample preparation techniques (*e.g*. Geisen *et al*., 1999; Herrle & Bollmann, 2004; Henderiks & Törner, 2006).

The standard simple smear-slide technique (*e.g*. Backman & Shackleton, 1983; Bown & Young, 1998) and the spray method (McIntyre *et al*., 1967; Bollmann *et al*., 1999) are two frequently used methods to prepare calcareous nannofossil microscope slides for light-microscopic analysis. Both techniques are quick, easy to perform and low-cost compared to other methods used for coccolith preparation (*e.g*. the random settling technique: Beaufort, 1991; Geisen *et al*., 1999). A recent comparative study between these two methods showed that the variance of the coccolith counts obtained in smear replicates is three to four times higher than the values obtained by counting spray replicates (Henderiks & Törner, 2006). The values obtained are apparently reproducible in repeated preparation only when using spray replicate slides. Combining results of coccolith morphometry and species abundance counts, the spray method was preferred for biometric studies (Henderiks & Törner, 2006).

The aims of this study were to re-evaluate the internal accuracy and reproducibility of quantitative coccolith assemblage counts for each preparation technique, to compare the species proportions that the two methods yield, and to observe any systematic difference between the methods. In addition, accuracy and reproducibility were tested for a commonly used semi-quantitative method (number of specimens per mm^2: Backman & Shackleton, 1983). Based on the obtained results, a fourth aim was to decide upon which method would be preferred for generating biostratigraphic and palaeoecological data in future high-resolution nannofossil investigations.

Sample #	Sample ID	Depth (mcd)
1	1218A-023X-04, 2-3cm	223.36
2	1218A-010H-01, 25-26cm	96.35
3	1218A-013H-03, 100cm	131.61
4	1218A-016H-06, 25-26cm	166.79
5	1218A-020H-01, 130cm	202.78
6	1218A-023X-05, 105cm	253.79

Table 1: Samples used in this study (ODP Leg 199, Hole 1218A); mcd = metres composite depth (depth in core)

2. Material and methods

2.1 Samples

A set of six samples (Table 1) was prepared using calcareous nannoplankton ooze from the Lower through Upper Oligocene of Ocean Drilling Program (ODP) Leg 199, Site 1218 (8°53.378'N; 135°22.00'W), located in the equatorial Pacific Ocean, in a water-depth of 4826m (Lyle *et al*., 2002). The samples were selected from well preserved sediments of Oligocene age and covered different stratigraphic intervals.

In order to provide comparable data with different methods, we tested the reproducibility and accuracy of relative coccolith species abundances obtained using the standard simple smear-slide and the spray preparation techniques. With each technique, 15 replicates of Sample #1 and five replicates each of Samples #2 - #6 were prepared.

2.2 Slide preparation

The preparation of the simple smear-slides for light-microscopic examination followed standard procedures, as described by Haq & Lohmann (1976), Backman & Shackleton (1983) and Bown & Young (1998), where smear-slide replicates were prepared from the raw sediment. Each smear replicate (15 in total) was subsampled from the 1cm^3 bulk sediment sample. A small fraction of sediment and a few drops of distilled water were placed onto a glass microscope slide. This was smeared thinly across the surface of the glass slide using the narrow side of a flat wooden toothpick, until a thin layer of rippled material was obtained. The slide was then dried rapidly on a hotplate. After drying, a coverglass was attached onto the glass slide using a mounting medium (AYAC). Air bubbles were removed by gentle pressure with a spatula. After cooling, washing away the excess sediment and labeling, the slide was ready for analysis under the light microscope. The whole procedure takes only a few minutes for preparing one smear-slide. Two series (X and Y) of replicate smear-slides were used in testing the semi-quantitative biostratrigraphic analysis.

Spray-slide preparation followed a procedure developed by McIntyre *et al*. (1967) and modified by Bollmann *et al*. (1999), but without spiking with microbeads. The preparation of the spray-slides was made in three series of five replicates for Sample #1 (15 in total) and one series of five replicates (five in total) for Samples #2 - #6 (Table 2). Before slide preparation, three subsamples, ranging from 0.0600 to 0.1351g sediment, were taken from Sample #1 (Table 3), and only one subsample was taken from Samples #2 - #6. For each subsample, the sediment was subsequently suspended in 10ml denatured alcohol, ultrasonified for about two minutes at 35kHz and further homogenised by gentle shaking. If the sediment was not fully suspended after two minutes, the ultrasonic treat-

		A	C	E	G	K	L	M	P
sample # 2	**N**	***Sm***	***Ru***	***Db***	***Cp***	***Dd***	***Cf***	***Hc***	***Cn***
SMEAR 1	**308**	29.8	0	0	6.1	19.4	43.8	0	0.6
SMEAR 2	**320**	28.1	0	0	5.3	14.3	51.6	0	0.6
SMEAR 3	**318**	29.8	0	0	4.7	13.5	50.9	0	0.9
SMEAR 4	**302**	28.1	0	0	3.6	18.2	46.6	0	0.3
SMEAR 5	**325**	26.1	0	0	3.6	20.6	48.6	0	0.6
mean		**28.38**	**0.00**	**0.00**	**4.66**	**17.2**	**48.3**	**0.00**	**0.6**
st.dev.		**1.53**	**0.00**	**0.00**	**1.09**	**3.14**	**3.20**	**0.00**	**0.21**
Sx-bar		**0.31**	**0.00**	**0.00**	**0.22**	**0.63**	**0.64**	**0.00**	**0.04**
95% CI		**0.60**	**0.00**	**0.00**	**0.43**	**1.23**	**1.25**	**0.00**	**0.08**
SPRAY 1	**305**	29.5	0	0	2.9	8.5	58.3	0	0.6
SPRAY 2	**304**	21.7	0	0	6.8	18	52.4	0.3	0.3
SPRAY 3	**316**	30	0	0	5	18.3	45.8	0.3	0.3
SPRAY 4	**315**	24.7	0	0	6	15.2	53.3	0	0.6
SPRAY 5	**347**	27.3	0	0	5.7	14.6	51.8	0	0.2
mean		**26.64**	**0.00**	**0.00**	**5.28**	**14.92**	**52.32**	**0.12**	**0.4**
st.dev.		**3.47**	**0.00**	**0.00**	**1.48**	**3.95**	**4.46**	**0.16**	**0.19**
Sx-bar		**0.69**	**0.00**	**0.00**	**0.30**	**0.79**	**0.89**	**0.03**	**0.04**
95% CI		**1.36**	**0.00**	**0.00**	**0.58**	**1.55**	**1.75**	**0.06**	**0.07**
sample # 3									
SMEAR 1	**324**	14.8	0	0.6	15.1	26.2	42.9	0	0.3
SMEAR 2	**306**	17.3	0	0.3	16	20.9	44.1	0.3	0.9
SMEAR 3	**338**	13.6	0	0.5	12.4	24.2	48.8	0	0.2
SMEAR 4	**309**	12.9	0	0	18.4	22.6	45.3	0	0.6
SMEAR 5	**303**	14.1	0	0	14.8	32.1	47.8	0	0
mean		**14.54**	**0.00**	**0.28**	**15.34**	**25.20**	**45.78**	**0.06**	**0.40**
st.dev.		**1.69**	**0.00**	**0.28**	**2.17**	**4.33**	**2.48**	**0.13**	**0.35**
Sx-bar		**0.34**	**0.00**	**0.06**	**0.43**	**0.87**	**0.50**	**0.03**	**0.07**
95% CI		**0.66**	**0.00**	**0.11**	**0.85**	**1.70**	**0.97**	**0.05**	**0.14**
SPRAY 1	**305**	15.4	0	0	13.1	26.2	44.2	0	0.9
SPRAY 2	**346**	17	0	0	14.1	28	40.1	0	0.5
SPRAY 3	**300**	15.6	0	0	12.3	26.6	44.6	0	0.6
SPRAY 4	**302**	16.5	0	0	15.8	32.1	43.7	0	0.6
SPRAY 5	**312**	15.7	0	0.3	12.5	29.1	41.6	0	0.6
mean		**16.04**	**0.00**	**0.06**	**13.56**	**28.40**	**42.84**	**0.00**	**0.64**
st.dev.		**0.68**	**0.00**	**0.13**	**1.43**	**2.37**	**1.92**	**0.00**	**0.15**
Sx-bar		**0.14**	**0.00**	**0.03**	**0.29**	**0.47**	**0.38**	**0.00**	**0.03**
95% CI		**0.27**	**0.00**	**0.05**	**0.56**	**0.93**	**0.75**	**0.00**	**0.06**
sample # 4									
SMEAR 1	**317**	20.5	0	1.5	11	8.2	57	0	0.3
SMEAR 2	**318**	23.5	0	1.2	9.4	14.4	50.9	0	0.3
SMEAR 3	**306**	15	0	0.6	11.7	8.1	63.7	0.3	0.3
SMEAR 4	**348**	16.9	0	0.8	8.9	11.4	61.2	0	0.5
SMEAR 5	**325**	16.3	0	0	11.6	9.8	61.5	0	0.6
mean		**18.44**	**0.00**	**0.82**	**10.52**	**10.38**	**58.86**	**0.06**	**0.40**
st.dev.		**3.49**	**0.00**	**0.58**	**1.29**	**2.62**	**5.07**	**0.13**	**0.14**
Sx-bar		**0.70**	**0.00**	**0.12**	**0.26**	**0.52**	**1.01**	**0.03**	**0.03**
95% CI		**1.37**	**0.00**	**0.23**	**0.51**	**1.03**	**1.99**	**0.05**	**0.06**
SPRAY 1	**305**	17.3	0	1.3	10.1	8.5	62.6	0	0
SPRAY 2	**305**	16.4	0	0	14.7	9.5	59	0	1.3
SPRAY 3	**302**	16.8	0.3	0	12.2	9.6	59.6	0	1.3
SPRAY 4	**324**	13.5	0	1.2	10.8	8.9	65.1	0	0.3
SPRAY 5	**310**	13.8	0	0.9	8.3	13.2	62.5	0	0.9
mean		**15.56**	**0.06**	**0.68**	**11.22**	**9.94**	**61.76**	**0.00**	**0.76**
st.dev.		**1.78**	**0.13**	**0.64**	**2.40**	**1.88**	**2.48**	**0.00**	**0.59**
Sx-bar		**0.36**	**0.03**	**0.13**	**0.48**	**0.38**	**0.50**	**0.00**	**0.12**
95% CI		**0.70**	**0.05**	**0.25**	**0.94**	**0.74**	**0.97**	**0.00**	**0.23**
sample # 5									
SMEAR 1	**302**	15.5	0.3	0.6	10.2	3.9	67.8	0.3	0.9
SMEAR 2	**312**	22.1	0	0	8.3	0.9	66	0.6	1.9
SMEAR 3	**342**	16.6	0.5	0.2	12.2	1.7	67.5	0.5	0.2
SMEAR 4	**323**	19.5	0.9	0.9	8.5	1.5	68.1	0	0.6
SMEAR 5	**346**	13	0	0	8.9	2	75.7	0	0.2
mean		**17.34**	**0.34**	**0.34**	**9.62**	**2.00**	**69.02**	**0.28**	**0.76**
st.dev.		**3.54**	**0.38**	**0.40**	**1.62**	**1.14**	**3.82**	**0.28**	**0.70**
Sx-bar		**0.71**	**0.08**	**0.08**	**0.32**	**0.23**	**0.76**	**0.06**	**0.14**
95% CI		**1.39**	**0.15**	**0.16**	**0.64**	**0.45**	**1.50**	**0.11**	**0.28**
SPRAY 1	**327**	11.9	0	1.5	10.7	4.5	70.6	0	0.6
SPRAY 2	**304**	13.4	0	0.6	8.8	2.9	73.3	0.3	0.3
SPRAY 3	**322**	13.7	0	1.5	12.4	3.1	69.8	0	0.3
SPRAY 4	**301**	15.2	0	0.9	8.6	2.6	71.7	0	0.6
SPRAY 5	**333**	15.9	0	1.2	10.8	3.6	68.1	0	0.3
mean		**14.02**	**0.00**	**1.14**	**10.26**	**3.34**	**70.70**	**0.06**	**0.42**
st.dev.		**1.57**	**0.00**	**0.39**	**1.58**	**0.74**	**1.96**	**0.13**	**0.16**
Sx-bar		**0.31**	**0.00**	**0.08**	**0.32**	**0.15**	**0.39**	**0.03**	**0.03**
95% CI		**0.62**	**0.00**	**0.15**	**0.62**	**0.29**	**0.77**	**0.05**	**0.06**
sample # 6									
SMEAR 1	**304**	15.1	1.9	12.8	29.6	0.6	35.1	2.6	1.9
SMEAR 2	**333**	12.6	1.5	12	26.4	1.2	43.5	2.1	0.6
SMEAR 3	**319**	12.2	0.3	9	34.1	1.5	39.1	2.1	1.2
SMEAR 4	**321**	10.9	0.9	14.3	32	1.5	38.8	1.5	0.3
SMEAR 5	**305**	11.8	0.9	15	28.5	2.2	35.4	4.9	0.9
mean		**12.52**	**1.10**	**12.62**	**30.12**	**1.40**	**38.38**	**2.64**	**0.98**
st.dev.		**1.57**	**0.62**	**2.35**	**3.00**	**0.58**	**3.41**	**1.32**	**0.61**
Sx-bar		**0.31**	**0.12**	**0.47**	**0.60**	**0.12**	**0.68**	**0.26**	**0.12**
95% CI		**0.62**	**0.24**	**0.92**	**1.18**	**0.23**	**1.34**	**0.52**	**0.24**
SPRAY 1	**309**	12.6	1.6	18.7	25.5	1.2	37.5	2.2	0.3
SPRAY 2	**311**	11.8	2.8	19.9	31.8	3.5	27.3	1.6	0.9
SPRAY 3	**336**	15.7	1.4	17.8	28.5	3.5	31.5	0.8	0.2
SPRAY 4	**313**	14.6	0.6	20.4	30.6	1.2	30	1.5	0.6
SPRAY 5	**322**	17	3.1	17.7	25.7	1.8	31.6	2.4	0.3
mean		**14.34**	**1.90**	**18.90**	**28.42**	**2.24**	**31.58**	**1.70**	**0.46**
st.dev.		**2.15**	**1.03**	**1.22**	**2.83**	**1.18**	**3.74**	**0.63**	**0.29**
Sx-bar		**0.43**	**0.21**	**0.24**	**0.57**	**0.24**	**0.75**	**0.13**	**0.06**
95% CI		**0.84**	**0.41**	**0.48**	**1.11**	**0.46**	**1.47**	**0.25**	**0.11**

Table 2 (right): Descriptive statistics for assemblage counts in five smear and five spray replicates of Samples #2 - #6. For explanation of codes, see Table 3 caption

Sample #1	N	A *Sm*	B *Sp*	C *Ru*	D *Rd*	E *Db*	F *Dh*	G *Cp*	H *Ce*	I *Ef*	J *Eo*	K *Dd*	L *Cf*	M *Hc*	N *Zb*	O *Bs*	P *Cn*	R Rest
SMEAR 1	**311**	10.2	8	0.3	2.2	3.8	11.2	27.3	1.6	0.6	1.6	0.6	27.9	0.6	0	0	0.3	0.3
SMEAR 2	**318**	12.5	4	0.6	3.7	4	11.9	21	0.9	0.3	2.5	0.3	33.6	1.5	0	0	2.5	0
SMEAR 3	**321**	15.2	6.5	1.2	4	3.7	9.6	21.8	1.5	0.9	2.8	1.2	29.5	0.9	0	0	0.6	0
SMEAR 4	**303**	11.5	2.6	0.6	3.3	4.2	14.8	23.1	1.3	1.3	0.9	0.3	32	0.9	0	0.6	1.6	0.3
SMEAR 5	**342**	12.5	4.9	0.8	2	5.5	16	21.3	2.3	1.4	1.7	0.8	26.9	1.1	0	1.1	1.4	1.1
SMEAR 6	**332**	11.4	3	1.8	3.6	2.4	10.2	22.5	1.5	1.5	2.7	1.8	34.9	0.6	0	0.3	1.2	0.3
SMEAR 7	**363**	14.9	4.6	0.2	6.6	4.9	14.6	18.5	1.3	0.2	0.5	1.9	29.2	0.2	0.8	0	1.1	0
SMEAR 8	**370**	11	5.9	0.2	0.5	4.8	16.4	20.5	1.3	1.3	2.1	1.3	27	0.8	0	0	1	0.2
SMEAR 9	**312**	14	4.5	0.6	6.2	4.2	12.7	18.6	0.9	0.9	1.3	0.3	34.9	0.6	0	0	1.6	0
SMEAR 10	**370**	9.4	3.7	0.8	4.8	4.3	16.2	19.1	1	0.5	2.4	0.8	32.7	2.4	0	0.5	0.8	0
SMEAR 11	**314**	12.7	3.8	0.9	4.7	5.4	16.8	17.5	0.9	0.3	3.1	2.5	27.3	0.9	0	0.3	0.9	0.3
SMEAR 12	**305**	15.4	4.2	0.6	4.2	3.2	16.7	20.9	0.6	0.3	0.9	1.6	27.8	0.3	0	0.3	0.6	0
SMEAR 13	**346**	16.1	4.6	0.2	4	2.8	15.6	22.2	0.2	0.5	0.8	0.5	29.7	0.8	0	0	1.1	0
SMEAR 14	**303**	11.5	2.6	0.3	4.9	6.2	19.8	20.7	0.9	1.6	0.9	0.9	24.4	2.6	0	1.6	0.3	0
SMEAR 15	**325**	15	1.8	0.6	4.3	5.5	18.4	26.6	0.9	0.9	0.9	0.6	26.1	2.4	0	0.3	1.2	0
mean		**12.89**	**4.31**	**0.65**	**3.93**	**4.33**	**14.73**	**21.44**	**1.14**	**0.83**	**1.67**	**1.03**	**29.59**	**1.11**	**0.05**	**0.33**	**1.08**	**0.17**
St. dev.		**2.09**	**1.61**	**0.43**	**1.55**	**1.07**	**3.00**	**2.74**	**0.49**	**0.49**	**0.86**	**0.67**	**3.30**	**0.77**	**0.21**	**0.47**	**0.57**	**0.29**
Sx-bar		**0.14**	**0.11**	**0.03**	**0.10**	**0.07**	**0.20**	**0.18**	**0.03**	**0.03**	**0.06**	**0.04**	**0.22**	**0.05**	**0.01**	**0.03**	**0.04**	**0.02**
95% CI		**0.27**	**0.21**	**0.06**	**0.20**	**0.14**	**0.39**	**0.36**	**0.06**	**0.06**	**0.11**	**0.09**	**0.43**	**0.10**	**0.03**	**0.06**	**0.07**	**0.04**
Series I 0.1315g																		
SPRAY 1	**310**	11.6	9	1.9	5.8	6.7	14.5	12.2	2.9	2.2	1.9	0.3	19.6	4.5	1.2	2.2	1.9	0.9
SPRAY 2	**336**	13.6	8.6	1.4	8	5	12.7	16.9	2	0.5	2.3	0.8	19.6	2.6	0	1.1	1.4	2.2
SPRAY 3	**334**	11.6	6.2	0.8	6.2	5.6	18.2	15.2	1.7	1.1	1.4	1.1	21.5	2.3	1.1	0.5	1.7	2.4
SPRAY 4	**328**	13.1	7	1.2	6.7	3	12.5	15.8	0.9	1.8	3.3	0.9	24.6	3.3	0.6	1.2	2.1	1.5
SPRAY 5	**321**	9.9	4.6	0.6	6.2	8	14.6	17.4	1.5	2.1	2.1	0.9	23.6	1.5	0.3	0.6	1.2	3.4
Series II 0.1002g																		
SPRAY 6	**321**	12.4	11	0.6	5.2	5.9	5.2	20.4	0.9	2.1	0.8	0.3	23.6	1.5	0.3	0	1.2	7.4
SPRAY 7	**315**	13	7.9	0.9	4.1	8.2	12.6	20	0.6	0.9	2.2	1.2	22.5	0.6	0	0	1.5	3.1
SPRAY 8	**316**	10.4	6.3	1.2	4.1	6.3	17.7	18	0.6	0.6	1.8	1.2	27.5	0.3	0	0	1.8	1.5
SPRAY 9	**326**	15	6.4	0.9	5.2	5.5	12.5	20.2	1.2	0.9	1.5	0.9	23	2.4	0.3	0	1.2	2.4
SPRAY 10	**306**	15.3	5.8	1.3	0.3	5.2	14	18.3	0.3	0.6	0.6	0	23.8	2.9	0.6	0	1.6	5.8
Series III 0.0600g																		
SPRAY 11	**319**	12.2	7.2	0.6	2.8	5	12.2	20	1.5	0.6	1.8	0.9	29	1.8	0	0.6	0.9	1.2
SPRAY 12	**304**	11.1	8.5	0.6	2.9	4.6	13.4	24.6	0.9	0.6	3.2	1.9	21.7	2.6	0	0	1.9	0.6
SPRAY 13	**316**	12.6	5	0.9	2.5	3.7	10.7	20.5	0.9	0.9	2.5	1.5	31.6	1.5	0	0	0.9	3.4
SPRAY 14	**328**	11.8	6.7	1.2	2.7	7	14.9	18.5	1.5	0.6	2.1	1.2	25.9	2.4	0	0.3	0.6	2.1
SPRAY 15	**307**	15.3	4.8	0.6	4.5	4.5	15.3	19.2	1.9	0.6	2.2	1.6	25.7	1.3	0	0	0.6	1.3
mean		**12.59**	**7.00**	**0.98**	**4.48**	**5.61**	**13.40**	**18.48**	**1.29**	**1.07**	**1.98**	**0.98**	**24.21**	**2.10**	**0.29**	**0.43**	**1.37**	**2.61**
St. dev.		**1.67**	**1.76**	**0.38**	**2.00**	**1.46**	**3.04**	**2.86**	**0.67**	**0.64**	**0.74**	**0.51**	**3.33**	**1.06**	**0.41**	**0.64**	**0.48**	**1.86**
Sx-bar		**0.11**	**0.12**	**0.03**	**0.13**	**0.10**	**0.20**	**0.19**	**0.04**	**0.04**	**0.05**	**0.03**	**0.22**	**0.07**	**0.03**	**0.04**	**0.03**	**0.12**
95% CI		**0.22**	**0.23**	**0.05**	**0.26**	**0.19**	**0.40**	**0.37**	**0.09**	**0.08**	**0.10**	**0.07**	**0.43**	**0.14**	**0.05**	**0.08**	**0.06**	**0.24**

Table 3: Descriptive statistics for calcareous nannofossil assemblage counts of 15 smear and three series of five spray replicate slides of Sample #1. Counting categories: A, *Sphenolithus moriformis*; B, *S. predistentus*; C, *Reticulofenestra umbilica*; D, *R. dictyoda*; E, *Dictyococcites bisectus*; F, *D. hesslandii*; G, *Coccolithus pelagicus*; H, *C. eopelagicus*; I, *Ericsonia formosa*; J, *E. obruta*; K, *Discoaster deflandrei*; L, *Cyclicargolithus floridanus*; M, *Helicosphaera compacta*; N, *Zygrhablithus bijugatus*; O, *Bramletteius serraculoides*; P, *Coronocyclus nitescens*; Rest, unidentified taxa

ment was repeated for another 30 seconds. The obtained suspension was then subsampled with a 2ml syringe and sprayed from ~30cm working distance, through small glass capillaries, onto a glass slide. Before each syringe subsample, the suspension was ultrasonified again for another 30 seconds to homogenise before being sprayed onto a new glass slide (see also Henderiks & Törner, 2006). A series of five replicate slides were sprayed from each suspension. Coverglasses were attached onto the dried glass slides using Norland Optical Adhesive (NOA61) under UV light.

2.3 Counting technique

All slides were analysed using a Leica DMLP polarising light-microscope at a magnification of 1000x. The counts of the nannofossil assemblages were performed on different fields of view (FOV) in all replicate slides. The counting was terminated when at least 300 specimens per slide were counted, so that species representing less than 3% of the assemblage would be included into the assemblage (*e.g.* Fatela & Taborda, 2002). In each slide, different FOV (three to eight for the smear- and five to 41 for the spray-slides) were analysed and counted first 'systematically', following predefined traverses along the slide, and then 'randomly', but still following a designated order along selected trajectories on each slide, in the following way: for the spray series the 'systematic' counting began in the lower right corner of the slide and continued upwards along the entire length of the slide, while for the smear series the counting was made on the thicker parts of the ripples, across the width of the slide. The 'random' counting began in the middle lower part of the spray slides, and every fifth FOV was counted along the length of the slide, while in the smear replicates, the counting started again on the thick part of a ripple (randomly chosen), across the width of the slide, and only every fifth FOV was considered. Only nannofossils that were completely within the FOV, and specimens which had their base-point within the FOV (*i.e.* nannofossils that are truncated at the top edge of the FOV; specimens truncated at the lower edge of the FOV were not included in the counts) were counted, in order to avoid a size bias.

Seventeen species (see Appendix) were identified in Sample #1 but we used only eight counting categories, which included the following species: *Sphenolithus moriformis (Sm), Reticulofenestra umbilica (Ru), Dictyococcites bisectus (Db), Coccolithus pelagicus (Cp), Discoaster deflandrei (Dd), Cyclicargolithus floridanus (Cf), Helicosphera compacta (Hc), Coronocyclus nitescens (Cn)* and one category labeled 'rest' (R). These eight species were counted also in Samples #2 - #6.

2.4 Biostratigraphic abundance counts

To test the reproducibility of semi-quantitative biostratigraphical analysis, all specimens of five selected species were counted from the fifteen smear replicates of Series X and Y, which represent two different series of replicates (Sample #1). The procedure involved the counting of *R. umbilica, E. formosa, D. deflandrei, H. compacta* and *C. nitescens* in a pre-selected area of the slide, in which each FOV had a relatively constant nannofossil density (Backman & Shackleton, 1983). All counts were made in a predetermined number of FOV (25). The data was then expressed relative to the unit area of slide examined (number of specimens per mm^2). To calculate the number of specimens per mm^2, the total number of specimens counted in each slide was divided by the total area,

$$Area = Pi\ (3.1415) * VFr\ (0.1) * VFr\ (0.1) * \#VF$$

where VFr = 0.1, radius in mm, and #VF = number of FOV counted.

2.5 Statistical methods

The statistical analyses were performed with MS *Excel* and *PhStat* software (Levine *et al.*, 2001). Chi-square tests for multiple samples were applied for comparing the proportion of different species between replicate slides in order to (1) determine the reproducibility of relative species abundances for both smear and spray methods, and (2) to examine whether the two methods produced equal species proportions. For all samples, the standard deviation and the 95% confidence of the mean species proportions were calculated.

3. Results

3.1 The internal reproducibility of the two methods

The descriptive statistics and raw proportion data for all six samples are given in Tables 2 and 3. In the chi-square test, for the first set of 15 replicates of Sample #1, nine counting categories, including abundant (34.9%) to very rare (1%) taxa, were taken into account. The chi-square tests were applied separately for smear- and spray-slides counted systematically or randomly.

The results of all chi-square tests are given in Table 4. For the 15 smear replicates counted systematically, the chi-square test for equality of proportion between slides generated a test statistic of 137.42, with 112 *df* and a p-value of 0.051. The same test for the randomly counted slides gave a chi-square value of 89.69, with 112 *df* and p-values of 0.94. These results indicate that the smear-slides did not differ with respect to species proportion among replicate slides.

The chi-square test for equality of proportion within the spray series counted systematically generated a test statistic of 150.32, with 112 *df* and p-values of 0.009, indicating that there is a difference between slides regarding the species proportion. For the randomly counted series, the chi-square test yielded a value of 123.74, with 112 *df* and a p-values of 0.211. The observed levels of significance of the chi-square test was greater than the chosen level of significance, alpha = 0.05. This means that spray-slides counted randomly did not differ significantly with respect to species proportion among replicate slides.

SAMPLE	SMEAR systematically		randomly		SPRAY systematically		randomly		REPLICATES N
	Chi 2	Pr	Chi 2	Pr	Chi 2	Pr	Chi 2	Pr	
#1	137.42	0.051	89.69	0.94	150.32	0.009	123.74	0.211	15
#1 Sy-Series I-III					57.77	1.23E-06	35.11	0.003	15
#1 Sm/Sy	80.30	1.05E-14					60.55	3.63E	15
#1 Series A			102.07	0.000					
#1 Series B			47.40	0.786					

SAMPLE	SMEAR randomly		SPRAY randomly		REPLICATES N	SMEAR vs. SPRAY randomly		REPLICATES N
	Chi 2	Pr	Chi 2	Pr		Chi 2	Pr	
#2	15.13	0.514	32.32	0.039	5	8.42	0.134	10
#3	20.74	0.653	10.25	0.96	5	13.21	0.039	10
#4	35.56	0.06	31.48	0.14	5	8.79	0.26	10
#5	46.96	0.013	15.03	0.919	5	29.43	0.00	10
#6	36.13	0.139	33.48	0.218	5	45.45	1.12E-07	10

Table 4: Chi-square tests for smear and spray replicates of Samples #1 - #6. Pr = probability; Sm = smear; Sy = spray

The next test was to analyse if the spray technique gives the same proportion of species between the combined three series of the five replicates, counted either systematically or randomly. The chi-square tests yield *p*-values = 1.23E-06 (spray slides counted systematically) and 0.003 (randomly counted), indicating that there is a statistically significant difference at the set 95% confidence level between the three spray series.

3.2 Comparability of the two methods: 15 smear *vs*. 15 spray replicates

To test if the two methods are comparable regarding the species proportion, the total number of coccoliths in 15 spray *vs*. 15 smear replicates was compared. The results of the chi-square tests (*p*-value = 1.05E-14 for the systematic counts and *p*-value = 3.63E-10 for the random counts) show that there is a statistically significant difference in the total number of species proportions between the two preparation techniques (Table 4).

A two by two contingency table (Table 5), representing the total number of abundant (*S. moriformis, D. bisectus, C. pelagicus, C. floridanus* and Rest) and rare species (*R. umbilica, D. deflandrei, H. compacta* and *C. nitescens*) was formed to check why the two techniques give statistically different species proportions. The chi-square tests generate a *p*-value = 0.001 for the systematically counted slides and 0.002 for the randomly counted ones, indicating again that the two methods give different results. The abundant species were present more frequently in the smear-slides than in the spray-slides, and *vice versa* for the rare species.

Systematically	abundant	rare	total
SPRAY	4523	**265**	4788
SMEAR	**4730**	205	4935
total	9253	470	9723
	Chi2(1)= 10.0702		Pr=0.001

Randomly	abundant	rare	total
SPRAY	4548	**254**	4802
SMEAR	**4711**	195	4906
total	9259	449	9708
	Chi2(1)= 9.5092		Pr=0.002

Table 5: Abundant vs. rare counting categories. Data table for equality of proportions

To investigate if the two methods give the same proportional distribution among the abundant and rare species, two contingency tables of five (abundant species) by two (spray and smear techniques), and four (rare species) by two were formed. Among the abundant species, the chi-square test yields a *p*-value = 2.76E-13 for the systematically counted slides and a *p*-value = 4.67E-10 for the randomly counted ones (Table 6a), indicating that there is a statistically significant difference between the two methods, considering the distribution among the abundant taxa. Among the rare species, the chi-square test yields a *p*-value = 0.036 for the systematically counted slides and a *p*-value = 0.542 for the randomly counted one (Table 6b), indicating that there is no difference between the two techniques regarding the distribution among the rare taxa.

3.3 Chi-square tests for Samples #2 - #6

For the second set of samples, the counts were made only randomly. Contingency tables of eight (counting categories) by five (replicate slides) were formed for each method, separately, to investigate if the species proportions within each technique differed significantly. The results of the chi-square tests (Table 4) indicate that the proportion of different species counted within both smear and spray slides do not differ significantly (except Sample #5 smear and Sample #2 spray) among the replicate slides prepared using the smear and spray techniques, consequently providing good reproducibility. On the contrary, the chi-square tests for the comparison of the total proportions of taxa in five spray- *vs*. five smear-slides in each sample indicate a significant statistical difference between spray and slide replicates (Table 4) in Samples #3, #5 and #6, while it revealed no significant differences for Samples #2 and #4.

3.4 Semi-quantitative biostratigraphy

The internal reproducibility of the dataset for biostratigraphical investigation, using the specimens/mm^2 method, was tested using a matrix of 15 (smear replicate

Table 6a

Systematically	*S. moriformis*	*D. bisectus*	*C. pelagicus*	*C. floridanus*	Rest	total
SPRAY	607	271	886	1162	1597	4523
SMEAR	637	216	1039	1461	1377	4730
total	1244	487	1925	2623	2974	9253
	Chi2(4)=64.855		Pr=2.76E-13			
Randomly						
SPRAY	627	273	911	1171	1566	4548
SMEAR	625	222	1011	1460	1393	4711
total	1252	495	1922	2631	2959	9259
	Chi2(4)=49.465		Pr=4.67E-10			

Table 6b

Systematically	*R. umbilica*	*D.deflandrei*	*H.compacta*	*C. nitescens*	total
SPRAY	49	50	103	63	265
SMEAR	34	53	57	61	205
total	83	103	160	124	470
	Chi2(3)=8.535		Pr=0.036		
Randomly					
SPRAY	55	37	91	69	252
SMEAR	41	38	62	54	195
total	96	75	153	123	447
	Chi2(3)=2.147		Pr=0.542		

Table 6: Raw data representing the (6a) most abundant species (*S. moriformis*, *D. bisectus*, *C. pelagicus*, *C. floridanus*, Rest) and (6b) the rare species (*R. umbilica*, *D. deflandrei*, *H. compacta*, *C. nitescens*) for each preparation method

slides) by five (counting categories) for each smear series. In Series X, which includes smear-slides used for all tests above for assemblage counts, the dataset shows a variable distribution of the five counting categories within the 15 smear-slides (chi-square test, *p*-value = 0.000), while in the second series, Y, of 15 additionally prepared smear replicates, the result of the chi-square test (*p*-values = 0.786) indicates a good reproducibility of the species within the smear-slides (Table 4).

4. Discussion

4.1 Reproducibility of the assemblage counts

Our first objective was to re-evaluate the internal accuracy and reproducibility of the relative coccolith assemblage counts for the spray and smear preparation techniques. Comparison of the systematically *vs*. randomly counted datasets produced from each method shows no statistical difference between them. This indicates a good reproducibility of proportion estimates for all counting categories using one or other method. However, when comparing the datasets of the spray *vs*. smear replicates, the counts reveal significantly different species proportions, indicating that the two preparation techniques are not statistically comparable regarding the species proportions.

4.2 Reproducibility of counts of the biostratigraphical markers

Counts of the selected biostratigraphical markers in the 15 smear replicates vary significantly in Series X. This variation can be explained by the counting of FOV that did not have a relatively constant nannofossil density, which is a primary requirement of the specimens/mm^2 method of Backman & Shackleton (1983). When counting FOV (*i.e*. 25) with a relatively constant (~50 coccoliths/FOV) nannofossil density (Series Y), the chi-square test reveals good reproducibility of the selected markers.

4.3 Accuracy and reliability of the counts

Figure 1 shows the mean species abundance (in %), and corresponding standard deviation, of the 15 smear and 15 spray replicates of Sample #1. Figure 2 illustrates the mean species abundances (in %), and corresponding standard deviation, for Samples #1 - #6 plotted against depth (ODP Leg 199, Hole 1218A), while Figure 3 shows the mean of specimens/mm^2 and standard deviation in smear Series X and Y. Inspection of the variance reveals that both techniques indicate similar results for the accuracy and reliability of the counts. Since no significant difference in the reproducibility of the proportion estimates within replicates of the same method was identified, both techniques are suitable for nannofossil assemblage analyses. Although there is a statistically significant difference between the three spray series (Section 3.1), it appears that, for the purposes set in quantitative nannofossil analyses, the results are consistent within each method.

4.4 Systematic differences between the methods

Another aim was to explain any systematic differences between the two preparation techniques. A first observation is that the rare taxa were encountered more frequently in the spray series than in the smear series (Table 6a) and *vice versa* for the abundant species (Table 6b). Similar observations were first made and discussed in Henderiks & Törner (2006). The rare taxa include relatively large coccoliths (*i.e*. *R. umbilica*, *D. deflandrei*, *H. compacta*). This study confirms that the spray method may overestimate larger nannofossil taxa, or that the smear method may underestimate the same (Henderiks & Törner, 2006). The dominant taxon, *C. floridanus* (intermediate in size), is enriched in the smear replicates (total mean value 29.8%, compared to 24.1% in the spray replicates), whereas the abundance of the subordinate taxa are about the same for either method.

The reproducibility and accuracy of the relative coccolith abundance estimates, based on the two preparation methods, depends on the concentration of the slides, the constant distribution of the sediment on the glass slide (only for the specimens/mm^2 method), and on the assumption that no size-dependent fractionation takes place during the preparation process. The results can be

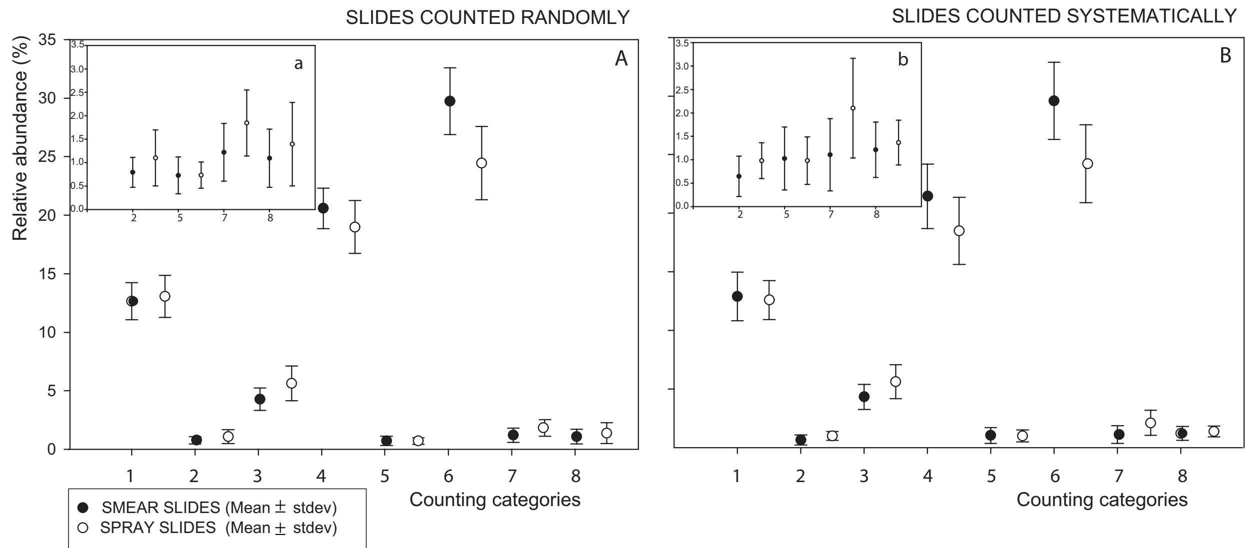

Figure 1: Mean species abundance (%) and corresponding standard deviation in the 15 smear and 15 spray replicates of Sample #1, counted (A) randomly and (B) systematically. Counting categories: (1) *S. moriformis*, (2) *R. umbilica*, (3) *D. bisectus*, (4) *C. pelagicus*, (5) *D. deflandrei*, (6) *C. floridanus*, (7) *H. compacta*, (8) *C. nitescens*. Subordinate taxa are shown in detail in inset figures (a, b)

biased due to the inhomogeneous distribution of particles during the preparation technique. The preparation procedure in both methods involved the mixing of the bulk sediment with water (smear) or denatured alcohol (spray). The spray method may possibly result in a more thorough mixing, due to the process of ultrasonification, and for

Figure 2: Mean species abundances (%) and corresponding standard deviation for Samples #1 - #6 plotted against depth (ODP Leg 199, Hole 1218A)

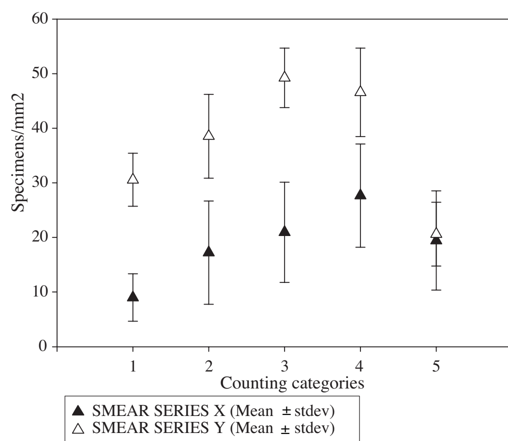

Figure 3: Specimens/mm^2 and standard deviation in smear Series X and Y. Counting categories: (1) *R. umbilica*, (2) *E. formosa*, (3) *D. deflandrei*, (4) *H. compacta*, (5) *C. nitescens*

this reason may result in a more random distribution of nannofossils than the smear method, which was supported by the chi-square statistics in the study of Henderiks & Törner (2006). However, Haq & Lohmann (1976) argued that many preparation techniques involving the disaggregation and suspension of the sample in liquids may sort coccoliths according to their hydrodynamic character and, thus, produce a bias in census data. To avoid this, they counted only smear-slides made directly from the raw sample.

Even if some size-sorting might occur during the smearing procedure, the chi-square tests of this study indicate no statistically significant differences in the estimates of the species proportions within each of the two methods. But, between the methods, we find a statistically significant difference.

A possible explanation for the statistically significant difference would be the fundamental difference between the two methods, the subsampling of the sediment: each replicate smear-slide was made from new bulk sediment, while each of the three series of five spray-slides was made from a new sediment suspension. However, considering routine nannofossil investigations, this type of subsampling would be the most common. Indeed, rarely are studies reported that systematically investigate replicate slides of one and the same subsample (*e.g.* Henderiks & Pagani, 2007). Therefore, we argue that the presented approach puts 'common practise' to the test in the best way.

The primary assumption was that no size-dependent fractionation took place during either slide preparation method and that both techniques give randomly distributed samples. Since we find a statistically significant difference between the methods, it is important to investigate if and how size-fractionation may have occurred during the preparation procedure, and if the sediment was not randomly distributed on the slides. In the process of smearing, some size-sorting may take place during the spreading of the sediment on the slide with the toothpick, and sporadic areas with lumpy clusters (where coccoliths of different size accumulate and cannot be identified) can be observed. In order not to bias the data, the FOV with very dense areas were not counted, thus potentially underestimating large coccoliths that form the 'nuclei' of such concentrated areas. A comparative test of relative abundance (%) counts between dense (100 specimens/FOV) and thin (20 specimens/FOV) areas on the same smear-slide indicates proportionally more large specimens in the dense ripples than in the thin areas of the slides (Table 7).

During the procedure of suspending the sediment in denatured alcohol and then spraying, although the aim is to obtain an even distribution of the material on the slides, coccoliths might be sorted according to their hydrodynamic properties and bias the data in the interval between mixing and spraying. In addition, the repeated ultrasonification process might increase fragmentation of coccoliths. However, the suspension is sampled immediately (3s) after the ultrasonic handling (60s). Stoll & Ziveri (2002) used Stokes' Law as an approximation for the relative settling times of different-sized particles. They demonstrated that the settling time for particles with estimated spherical diameter (ESD) >6.0μm is 30 minutes in ethanol, and that species are well separated spatially in the density-stratified settling-column only after five hours. These settling experiments demonstrate that the coccoliths do not sort that quickly.

Comparing the five replicates of each spray series, a pattern of progressively denser overall concentration of coccoliths on the slide, toward the last prepared slide of each series, was observed, representing a denser suspension which may either be due to evaporation of the ethanol medium, or some degree of settling. This possible size-biasing might be avoided by preparing spray-slides, from a single suspension per sediment sample. The many steps involved in the preparation of the spray-slides, and greater interaction with the sediment (sampling, ultrasonification, spraying) might result in an increased possibility to bias the census data, more so than using the smear tech-

	% relative abundance									
large specimens	1d	2d	3d	4d	5d	1t	2t	3t	4t	5t
D. bisectus	**22.7**	**20.0**	**19.7**	**26.8**	**28.5**	18.7	15.5	18.9	16.9	16.6
C. pelagicus	**20.1**	**19.7**	**24.0**	**22.1**	**21.8**	23.0	21.8	23.2	22.0	23.9
D.deflandrei	**0.3**	**3.5**	**2.0**	**2.0**	**1.5**	0.3	1.3	0.7	1.2	0.3
H. compacta	**1.0**	**1.9**	**1.0**	**2.0**	**2.3**	2.3	1.0	1.0	0.6	0.7
R. umbilica	**0.6**	**2.3**	**1.7**	**1.1**	**1.5**	1.0	1.0	1.0	1.0	0.3
small specimens										
C. floridanus	**29.9**	**31.6**	**29.0**	**26.5**	**26.5**	35.0	40.3	36.1	38.0	39.9
S. moriformis	**24.7**	**20.6**	**22.0**	**19.0**	**17.7**	19.3	18.5	18.9	19.5	17.6
C. nitescens	**0.6**	**0.3**	**0.7**	**0.6**	**0.3**	0.3	0.7	0.3	0.6	0.7

Table 7: Relative abundance (%) of nannofossils counted in dense *vs*. thin areas of five smear-slides (d = counted on the dense area of the slides: 100 specimens/FOV; t = counted on the thin area of the smear-slide: 20 specimens/FOV)

nique, where slides were made directly from the raw sample. Future experimental setups using a mixture of different known-sized granules and coccoliths are needed to test the various reasons for variability using the spray technique.

5. Conclusions

Assessment of the reproducibility and accuracy of semi-quantitative nannofossil assemblage counts is important in data acquisition, interpretation and comparison between various studies and methodologies. Our study indicates that both smear and spray techniques are suitable for generating palaeoecological data, as no statistically significant differences in the proportion of taxa appear between replicate slides, using one or other technique. However, we confirm a systematic difference between the techniques (Henderiks & Törner, 2006). Larger coccoliths may be overestimated in the spraying preparation by mechanical sorting processes during the suspension or spraying process, because sprayed slides consistently contain more large nannofossils than smear-slides. Alternatively, the pre-selection of FOV to count on rippled smear-slides may underestimate numbers of larger coccoliths because of sorting on the slide, with relatively more large nannofossils within very dense ripples. The specimens/mm^2 method gives good reproducibility of the abundances of selected markers when counting FOV with a relatively constant nannofossil density.

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References

Backman, J. & Shackleton, N.J. 1983. Quantitative biochronology of Pliocene and early Pleistocene calcareous nannofossils from the Atlantic, Indian and Pacific oceans. *Marine Micropaleontology*, **8**: 141-170.

Beaufort, L. 1991. Adaptation of the random settling method for quantitative studies of calcareous nannoplankton. *Micropaleontology*, **37**: 415-418.

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

Bown, P. & Young, J. 1998. Techniques. *In*: P.R. Bown (Ed.). 1998. *Calcareous Nannofossil Biostratigraphy*. Chapman & Hall, Cambridge: 16-28.

Fatela, F. & Taborda, R. 2002. Confidence limits of species proportions in microfossil assemblages. *Marine Micropaleontology*, **45**: 169-174.

Geisen, M., Bollmann, J., Herrle, J.O., Mutterlose, J. & Young, J.R. 1999. Calibration of the random settling technique for calculation of absolute abundances of calcareous nannoplankton. *Micropaleontology*, **45**: 437-442.

Haq, B.U. & Lohmann, G.P. 1976. Early Cenozoic calcareous nannoplankton biogeography of the Atlantic Ocean. *Marine Micropaleontology*, **1**: 119-194.

Henderiks, J. & Pagani, M. 2007. Refining ancient carbon dioxide estimates: Significance of coccolithophore cell size for alkenone-based *p*CO2 records. *Paleoceanography*, **22**: PA3202, 1-12.

Henderiks, J. & Törner, A. 2006. Reproducibility of coccolith morphometry: Evaluation of spraying and smear slide preparation techniques. *Marine Micropaleontology*, **58**: 207-218.

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

Levine, D., Ramsey, P. & Smidt, R. 2001. *Applied Statistics for Engineers and Scientists Using Microsoft Excel® and MINITAB®*. Prentice Hall, Upper Saddle River, New Jersey 07458: 671pp.

Lyle, M.W., Wilson, P.A., Janecek, T.R. *et al*., 2002. Leg 199 summary. *Proc. ODP, Initial Reps*, **199**: 1-125.

McIntyre, T.C., Bé, A.W.H. & Prekistas, R. 1967. Coccoliths and the Pliocene-Pleistocene boundary. *In*: M. Sears (Ed.). *Progress in Oceanography*. Pergamon, New York: 3-25.

Perch-Nielsen, K. 1985. Cenozoic calcareous nannofossils. *In*: H.M. Bolli, J.B. Saunders & K. Perch-Nielsen (Eds). *Plankton Stratigraphy*, **1**. Cambridge University Press, Cambridge: 427-554.

Stoll, H. & Ziveri, P. 2002. Separation of monospecific and restricted coccolith assemblages from sediments using differential settling velocity. *Marine Micropaleontology*, **46**: 209-221.

Appendix - taxon list

The taxonomy used here follows Perch-Nielsen (1985). Calcareous nannofossils encountered in this study are listed in alphabetical order.

Bramletteius serraculoides Gartner, 1969a
Coccolithus eopelagicus (Bramlette & Riedel, 1954) Bramlette & Sullivan, 1961
Coccolithus pelagicus (Wallich, 1877) Schiller, 1930
Coronocyclus nitescens (Kamptner, 1963) Bramlette & Wilcoxon, 1967
Cyclicargolithus floridanus (Roth & Hay *in* Hay *et al*., 1967) Bukry, 1971
Dictyococcites bisectus (Hay, Mohler & Wade, 1966) Bukry & Percival, 1971
Dictyococcites hesslandii (Haq, 1966) Haq & Lohman, 1976
Discoaster deflandrei Bramlette & Riedel, 1954
Ericsonia formosa (Kamptner, 1963) Haq, 1971
Ericsonia obruta Perch-Nielsen, 1971
Helicosphaera compacta Bramlette & Wilcoxon, 1967
Reticulofenestra dictyoda (Deflandre *in* Deflandre & Fert, 1954) Stradner *in* Stradner & Edwards, 1968
Reticulofenestra umbilica (Levin, 1965) Martini & Ritzkowski, 1968
Sphenolithus moriformis (Brönnimann & Stradner, 1960) Bramlette & Wilcoxon, 1967
Sphenolithus predistentus Bramlette & Wilcoxon, 1967
Zygrhablithus bijugatus (Deflandre *in* Deflandre & Fert, 1954) Deflandre, 1959
Unidentified counting category (Rest)