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Determining the absolute abundance of dinoflagellate cysts in recent marine sediments: The *Lycopodium* marker-grain method put to the test

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ABSTRACT

Absolute abundances (concentrations) of dinoflagellate cysts are often determined through the addition of *Lycopodium clavatum* marker-grains as a spike to a sample before palynological processing. An inter-laboratory calibration exercise was set up in order to test the comparability of results obtained in different laboratories, each using its own preparation method. Each of the 23 laboratories received the same amount of homogenized splits of four Quaternary sediment samples. The samples originate from different localities and consisted of a variety of lithologies. Dinoflagellate cysts were extracted and counted, and relative and absolute abundances were calculated. The relative abundances proved to be fairly reproducible, notwithstanding a need for taxonomic calibration. By contrast, excessive loss of *Lycopodium* spores during sample preparation resulted in non-reproducibility of absolute abundances. Use of oxidation, KOH, warm acids, acetolysis, mesh sizes larger than 15 µm and long ultrasonication (>1 min) must be avoided to determine reproducible absolute abundances. The results of this work therefore indicate that the

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dinoflagellate cyst worker should make a choice between using the proposed standard method which circumvents critical steps, adding *Lycopodium* tablets at the end of the preparation and using an alternative method.

1. Introduction

Dinoflagellate cyst concentrations are an important component of paleoceanographical studies (e.g. Pospelova et al., 2006; González et al., 2008) and can be determined using the volumetric method (e.g. Dale et al., 2002; Holzwarth et al., 2007). In general, dinoflagellate cyst concentrations are calculated by adding a known amount of exotic markers or a "spike" to every sample according to the method described by Stockmarr (1971). The marker commonly used is *Lycopodium clavatum* Linnaeus (Stag's Horn Clubmoss or Ground Pine).

As noted by Lignum et al. (2008), the so-called 'standard' palynological processing methods are still very variable in terms of initial sample sizes, type and concentration of acids, sieve material and mesh size, sonication time and strength, number of decanting cycles and use of heavy liquid separation. This is also apparent in reviews of the preparation techniques for extraction of dinoflagellate cysts given by Wood et al. (1996) and more recently by Riding and Kyffin-Hughes (2004). However, critical evaluation of the effect of different laboratory procedures on the marker grain technique for obtaining dinoflagellate cyst concentration has so far never been attempted. Although it has been reported that several processing methods such as sonication and chemical treatments can inflict damage on organic-walled microfossils to a certain extent (e.g. Schrank, 1988; Hodgkinson, 1991), the effect on palynomorph concentrations remain unknown.

This study aims to test the reproducibility of the marker-grain method, in order to understand the discrepancies in the results following different preparation techniques. Similar efforts to test the reproducibility of specific laboratory techniques have been done for other microfossil groups: benthic and planktonic foraminifera (Zachariasse et al., 1978), diatoms (Wolfe, 1997), nannofossils (Herrle and Bollman, 2004) and their biomarkers (Rosell-Melé et al., 2001). It is therefore timely to carry out a similar exercise with dinoflagellate cysts.

Surface sediment samples from four localities (North Sea, Celtic Sea, NW Africa and Benguela) were sent to 23 laboratories. The samples were processed using the palynological techniques routinely used in these laboratories. An equal amount of *Lycopodium* tablets, all from the same batch, were added to each sample. The reproducibility of both absolute and relative abundances for dinoflagellate cysts is here put to test, and has resulted in a proposal of recommendations for a standardized method to determine absolute abundances of Quaternary dinoflagellate cysts with the marker-grain method. Two laboratories used the volumetric method (Dale, 1976) for comparison purposes. This study focuses additionally on whether it is necessary to count 300 or 400 dinoflagellate cysts and on taxonomy, since notable interlaboratorial differences in nomenclature were recorded.

2. Material and methods

Late Quaternary surface sediment samples from four sites with different lithologies were used by the 23 different laboratories involved in the project. The North Sea sample consisted of a homogenized surface sediment taken using a Reineck boxcorer (51.47°N, 3.48°E, 10 m water depth). The Celtic Sea sample was assembled through mixing multi-corer samples from Station 8, collected during several time slots from the Celtic Sea (51.05°N, 5.83°W, 86 m water depth) (Marret and Scourse, 2002). The sample from Northwest Africa was a mixture of multicores GeoB9504-4 (15.87°N, 16.67°W, 43 m water depth) and GeoB9503-3 (16.07°N, 16.65°W, 50 m water depth). The Benguela sample consists of a mixture of sediment samples collected offshore Walvis Bay, at a water depth of about 200 m during Meteor cruise M63/2. Sample details are given in Table 1. Each laboratory was given a number, followed by a letter when the laboratory used more than one processing method. Laboratory identification and numbers were kept anonymous. A brief overview of the methods used is described in Sections 2.1–2.5. A special variation of this method is detailed in Section 2.6 and the volumetric method is detailed in Section 2.7. Details of the methods used are given in the Supplementary data.

Homogenization was done using the quartile method. The samples were oven-dried at a temperature of 58 °C for 24 h. The *Lycopodium* spore tablets used are produced and distributed by the Subdepartment of Quaternary Geology, University of Lund, Sweden (http://www.geol.lu.se/kvg/eng/). Ten *Lycopodium clavatum* tablets of batch 483216, ($X = 18.583$ per tablet, $s = \pm 1708$), were dispatched with the samples, and a fixed number of tablets was added by each laboratory to each sample.

2.1. Chemical treatment

Hydrochloric acid (HCl) with a concentration of 6.5–36% was added for the removal of carbonate. Some 20 to 300 ml was used depending on the intensity of the reaction. Cold HCl was used in most of the cases, although some laboratories used hot HCl with a temperature ranging between 42 and 80 °C. Afterwards, the residue was left to settle (15 min to 42 h). Laboratories that used short settle times at this step, used centrifugation or sieving to concentrate the sample. For centrifugation, the rotation speed used varied between 1900 and 3500 rpm, and lasted between 5 s to 10 min.

Demineralised or distilled water was used for rinsing until pH reached more neutral values of 5 to 7. One to 5 decanting cycles with intervals of 3 to 24 h were needed depending on HCl concentrations used. To avoid losing residue during decanting, some laboratories used centrifugation for concentration of the residue. Extensive rinsing is necessary for the removal of Ca^{2+}, to avoid calcium fluoride (CaF_2) precipitation during HF treatment. A few laboratories used KOH for neutralization (laboratory 2: 1% KOH and laboratory 18b: 10% KOH).

The siliciclastic component of the samples was removed by adding 10 to 250 ml of hydrofluoric acid (HF) with a concentration ranging from 19% to 70%. Commonly a concentration between 40 and 50% was used. All laboratories used cold HF, except laboratories 12 (42 °C), 2 (50 °C), 6 (60 °C), 10 (70 °C) and 23 (80 °C). Settling times varied between 12 and 144 h. A few laboratories repeated the HF treatment up to 3 times before all silicates were removed.

Before neutralisation, about 10 to 300 ml HCl with a concentration of 6.5 to 36 vol.% was added for the removal of formed fluorosilicates.

Table 1
Description of the samples.

Sample	Lithology	Dry weight (g)	Number of tablets added	# spores added	St dev spores
North Sea	Fine-medium sand	10	3	55,749	2959
Celtic Sea	Fine silty sand	10	1	18,583	1708
NW Africa	Clay	2	2	37,166	2416
Benguela	Clay	1	4	74,332	3417

Mostly cold HCl was used, although some laboratories used hot HCl with a temperature ranging between 42 and 100 °C. The following settling time varied between 15 min and 72 h. Again, laboratories that used short settling times, used centrifugation. The sample was subsequently rinsed with distilled water, until pH reached 5–7. The rinsing took 1 to 6 decanting cycles with intervals of 3 to 24 h, depending on the concentrations used. Again, to avoid losing residue during decanting, some laboratories used centrifuging for the concentration of the samples. One laboratory used KOH for the neutralisation (laboratory 2: 1%). A few laboratories skipped the second HCl treatment and proceeded directly to the rinsing with distilled water until pH reached values of 5–7. Several of these laboratories used centrifuging and/or sieving for concentration of the samples. During rinsing toxic HF was decanted and removed.

One laboratory (laboratory 22b) oxidised three of the samples (excluding the North-West Africa sample) with Schulze's solution (70% nitric acid saturated with potassium chlorate).

2.2. Mechanical treatment

Heavy liquid separation for the removal of heavy minerals was carried out by a few laboratories. Labs 10 and 16 used sodium polytungstate (SPT) at specific densities to isolate the palynological fractions.

Between 13 and 1800 s sonication was used to break down organic matter aggregates by some laboratories. Most laboratories used sonic baths (Branson™, Sonimasse™, Sonicor™, Eurolab™). Laboratory 8 used a standard oscillating sensor.

2.3. Sieving

Some laboratories pre-sieved before the chemical treatment for the elimination of the coarse fraction (mesh sizes of 100, 106, 120 and 150 µm) and/or fine fraction (mesh sizes of 10, 11 and 15 µm). All the laboratories added the *Lycopodium* tablets before pre-sieving, except laboratory 23.

Sieving after the chemical treatment was used to remove the fine fraction from the residue. Calgon (sodium hexametaphosphate) was used to disaggregate the material in a few cases. The sieve mesh sizes used varied from 6 to 20 µm, and meshes were made of nylon, polyester, polymer or steel. The devices used were hand, mechanical and water pressure pumps. Some laboratories sieved without using a pump.

Table 2
Average percentage of the different taxa in the four samples.

Species name	North Sea	Celtic Sea	NW Africa	Benguela
Round brown cysts (RBC)	35.8 ± 16.0	10.0 ± 7.7	3.4 ± 2.3	62.7 ± 17.0
Spiny brown cysts (SBC)	15.5 ± 12.5	1.7 ± 3.3	2.3 ± 2.4	8.5 ± 8.5
cysts of *Alexandrium* spp.	0.2 ± 0.3	0.5 ± 0.9	–	0.1 ± 0.5
cysts of *Gymnodinium* spp.	0.3 ± 0.6	0.3 ± 0.6	0.0 ± 0.1	0.0 ± 0.1
Stelladinium spp.	0.3 ± 0.3	0.2 ± 0.2	0.3 ± 0.3	0.1 ± 0.4
Lejeunecysta spp.	9.5 ± 12.0	1.5 ± 1.6	0.4 ± 0.5	1.4 ± 1.6
Selenopemphix spp.	5.5 ± 1.7	4.8 ± 2.1	1.0 ± 0.6	6.5 ± 6.3
Tuberculodinium vancampoae	0.0 ± 0.1	–	0.1 ± 0.3	0.0 ± 0.1
Polykrikos spp.	6.9 ± 3.5	5.7 ± 3.8	1.2 ± 0.8	1.1 ± 0.8
Xandarodinium xanthum	0.2 ± 0.3	0.1 ± 0.1	0.1 ± 0.2	0.0 ± 0.1
Dalella chathamense	–	–	–	0.0 ± 0.1
Extremely sensitive cysts (total)	74.3 ± 7.4	24.8 ± 11.2	15.4 ± 8.2	80.6 ± 9.9
Lingulodinium machaerophorum	1.5 ± 2.5	0.7 ± 0.9	86.2 ± 4.7	0.2 ± 0.5
Operculodinium spp.	2.8 ± 1.9	12.3 ± 3.7	0.5 ± 0.7	8.4 ± 6.6
Pyxidinopsis reticulata	0.0 ± 0.2	–	–	–
Spiniferites spp.	9.8 ± 3.5	51.8 ± 10.7	3.3 ± 1.1	5.5 ± 3.2
Quinquecuspis concreta	3.3 ± 2.1	2.3 ± 2.0	0.1 ± 0.1	1.0 ± 1.5
Trinovantedinium applanatum	0.2 ± 0.4	1.2 ± 1.0	0.2 ± 0.3	0.3 ± 0.4
Votadinium spp.	5.8 ± 6.6	0.5 ± 0.7	0.0 ± 0.1	0.7 ± 0.7
Moderately sensitive cysts (total)	23.6 ± 7.2	68.9 ± 10.7	90.3 ± 4.2	16.2 ± 9.7
Nematosphaeropsis labyrinthus	0.0 ± 0.1	0.0 ± 0.1	0.1 ± 0.1	2.1 ± 2.0
Impagidinium spp.	0.3 ± 0.6	0.15 ± 0.3	0.0 ± 0.1	0.0 ± 0.1
Operculodinium israelianum	0.2 ± 0.2	0.0 ± 0.1	0.4 ± 0.7	0.4 ± 0.7
Pentapharsodinium dalei	0.4 ± 0.5	2.6 ± 3.5	0.0 ± 0.1	0.2 ± 0.5
Polysphaeridium zoharyi	0.4 ± 0.6	0.1 ± 0.3	0.1 ± 0.5	0.2 ± 0.7
Ataxiodinium choane	0.0 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	–
Bitectatodinium spp.	0.6 ± 1.1	3.3 ± 2.0	0.1 ± 0.2	0.2 ± 0.6
Resistant cysts (total)	0.5 ± 0.6	6.2 ± 3.8	0.7 ± 0.9	3.1 ± 2.5

2.4. Staining and mounting of the slides

Staining with a colouring agent enhances contrast for optical microscopy and can be used for the detection of pre-Quaternary specimens (Stanley, 1966). Safranin-O, Fuchsin or Bismark Brown was used by a few laboratories. Not every laboratory stained the residue. Finally a few drops of a copper sulphate solution, thymol or phenol were often added to the residue for the inhibition of fungal growth.

Slides were mounted on a heated metal plate (65 °C) using a pipette, by strewing using a spatula or a mix of both methods. The mounting medium was usually glycerin jelly, but sometimes thymol, Elvacite, Eukitt, UV adhesive, or Canada balsam was used. Although sealing is not *per se* necessary (Poulsen et al., 1990), nail polish or

Plate I. *Polykrikos schwartzii* extracted from the North Sea sample using different methodologies. Labs are sorted from high (upper left corner) to low abundances (lower right corner).

1. Lab 1a.
2. Lab 20a.
3. Lab 13.
4. Lab 12.
5. Lab 19.
6. Lab 2.
7. Lab 11.
8. Lab 21a.
9. Lab 21b.
10. Lab 22a.
11. Lab 10a.
12. Lab 18b.
13. Lab 1b.
14. Lab 16.
15. Lab17.
16. Lab 10b.
17. Lab 18a.
18. Lab 5.
19. Lab 4.
20. Lab 22b, oxidized. All scale bars are 20 µm.

paraffin wax was used to seal the slides to protect the residue from degradation by dehydration.

2.5. Counting of the palynomorphs and calculation of absolute abundances

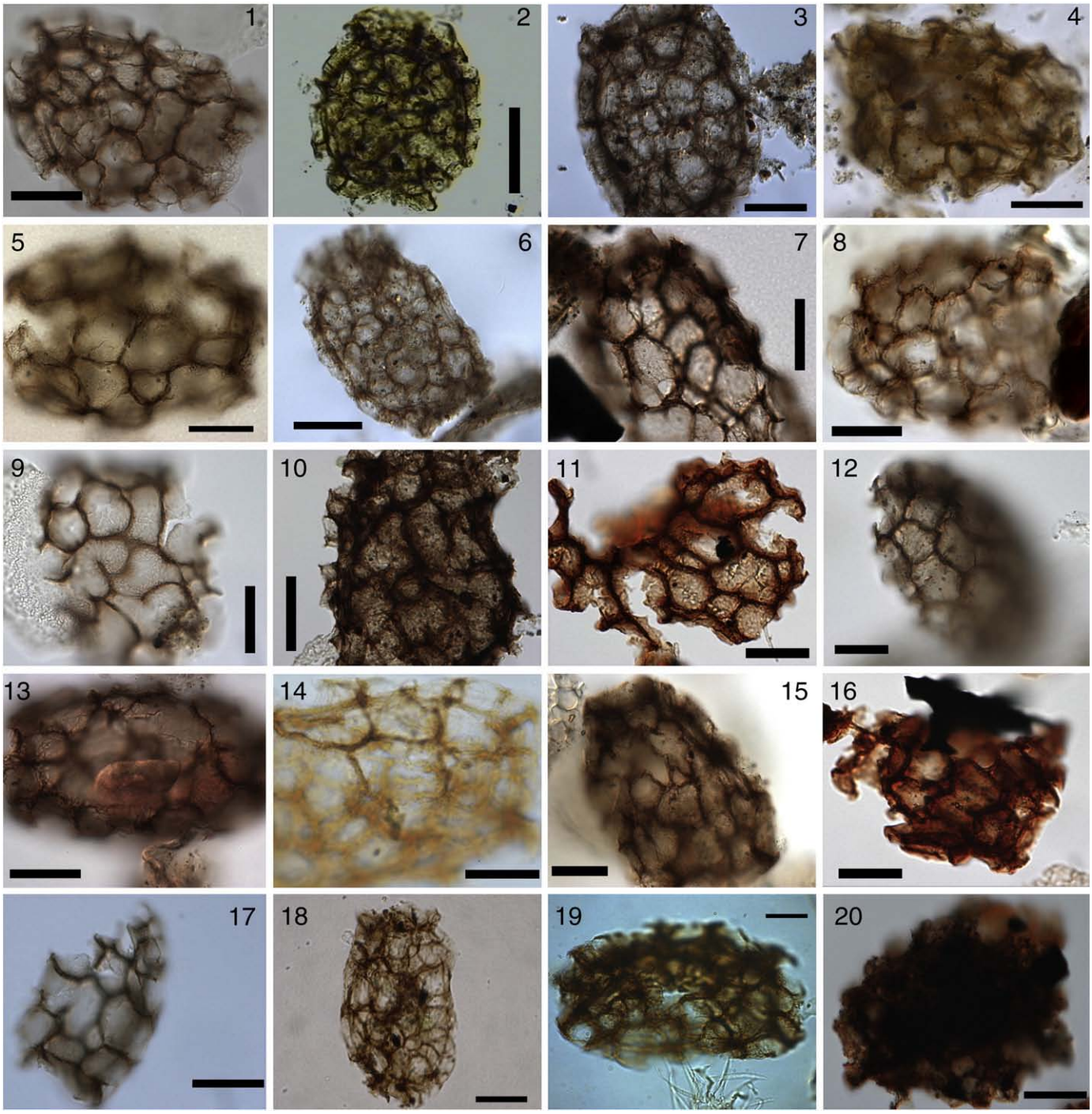
Dinoflagellate specimens were counted only when they comprised at least half of a cyst. The same criterion was used for other palynomorphs, also counted by some of the laboratories. Initially 300 dinoflagellate cysts were counted, and subsequently an extra 100 specimens were added. The purpose was to check whether it is necessary to count 300 or 400 dinoflagellate cysts to obtain representative relative and absolute abundances. Indeterminate dinoflagellate cysts were grouped as Indeterminate spp., and were not taken into account for the calculation of the relative abundances, since every observer had a different concept of what counts as an indeterminate dinoflagellate cyst, and this would introduce observer bias into the relative abundances. Raw counts together with a summary of the methodology are available as supplementary data to this article. Taxonomy follows Fensome and Williams (2004).

Absolute abundances of dinoflagellate cysts were calculated following the equation by Benninghoff (1962):

$$c = \frac{d_c \times L_t \times t}{L_c \times w}$$

where

c concentration = number of dinoflagellate cysts/gram dried sediment.



d_c number of counted dinoflagellate cysts
L_t number of *Lycopodium* spores/tablet
t number of tablets added to the sample
L_c number of counted *Lycopodium* spores
w weight of dried sediment (g)

Maher (1981) devised an algorithm to calculate confidence limits on microfossil concentrations. A slight correction to this algorithm was made, since the current study used sediment weight instead of sediment volume. The confidence limits calculated based on this algorithm have a 0.95 probability ($Z = 1.95$). It should be noted that these confidence limits are similar to the total error on concentration proposed by Stockmarr (1971); (Appendix B). These confidence limits can then be used in a statistical test to check whether microfossil concentrations are the same in two different samples (Maher, 1981). To investigate the reproducibility of results from the different laboratories, the coefficient of variation (or relative standard deviation) of all counts of a particular sample can be compared. Ideally, the results should fall within the confidence limits of Maher (1981), and thus the coefficient of variation calculated from these confidence limits can be used as a comparison.

2.6. Special methods: the maceration tank method (with HF) and the washing machine method (without HF)

The maceration tank method (Poulsen et al., 1990; Desezar and Poulsen, 1994) was used for HF treatment by laboratory 20a. Other processing steps are similar to those used by the other laboratories and are detailed in Poulsen et al. (1990) and Desezar and Poulsen (1994). Each sample is tightly wrapped in filter cloth (25 cm × 25 cm) with a mesh size of 10 μm, and the filter bags are packed in rubber foam for protection. The samples are placed inside the maceration tank and HF is conducted to the tank in PVC tubes. The samples are treated with cold HF for 7–8 days, after which the HF is drained out through a bottom-stop cock and led via PVC tubes directly to a waste-container for used hydrofluoric acid.

With the washing machine method, used by laboratory 20b, no HF is used. Each sample is tightly wrapped in filter cloth (25 cm × 25 cm) with a mesh size of 10 μm and the filter bags are packed in rubber foam for protection. The samples are washed in a standard household washing machine with a standard household washing powder, after which carbonates are removed with citric acid at 65 °C. Next the samples are again given a normal wash with a standard household washing powder. Finally the remaining minerals are removed by heavy

Plate II. *Polykrikos schwartzii* extracted from the Celtic Sea sample using different methodologies, sorted from high absolute abundances (upper left corner) to low absolute abundances (lower right corner).

1. Lab 14.
2. Lab 1a.
3. Lab 13.
4. Lab 3.
5. Lab 19.
6. Lab 12.
7. Lab 1b.
8. Lab 15b.
9. Lab 1c.
10. Lab 21b.
11. Lab 21a.
12. Lab 11.
13. Lab 5.
14. Lab 4.
15. Lab 16.
16. Lab 23.
17. Lab 17.
18. Lab 18a.
19. Lab 20a.
20. Lab 2. All scale bars are 20 μm.

Plate III. *Lingulodinium machaerophorum* extracted from the NW Africa using different methodologies, sorted from high (upper left corner) to low absolute abundances (lower right corner). (see on page 244)

1. Lab 11.
2. Lab 1a.
3. Lab 14.
4. Lab 13.
5. Lab 19.
6. Lab 10b.
7. Lab 21a.
8. Lab 1b.
9. Lab 12.
10. Lab 17.
11. Lab 21b.
12. Lab 6.
13. Lab18a.
14. Lab 18b.
15. Lab 1c.
16. Lab 15b.
17. Lab 22a.
18. Lab 4.
19. Lab 5.
20. Lab 20b.
21. Lab 16.
22. Lab 8.
23. Lab 23.
24. Lab 3. All scale bars are 20 μm.

liquid separation. This method removes the amorphous material very efficiently. Furthermore, since HF is not used, siliceous constituents (e.g. diatoms) are not destroyed. Heavy liquid separation with zinc dibromide ($ZnBr_2$) was used at densities of 2.3, 2.0 and 1.8 g/ml to remove heavy minerals. In order to test the influence of the specific density of the $ZnBr_2$, the NW African sample from laboratory 20b, was separated using heavy liquid densities of 1.8, 2.0 and 2.3 g/ml.

2.7. Volumetric method

For comparison with the marker-grain method, the volume aliquot method was performed by laboratories 6 and 8, following Dale (1976). This method was not used for the North Sea sample because of the difficulty associated with counting a fixed volume of this sample with very low abundances.

3. Results

3.1. Relative abundance of dinoflagellate cysts

Quantitative and qualitative disparities between assemblages recorded by the laboratories may be due to the different processing methods. It is obvious that aggressive agents could destroy the more sensitive cysts. To check this dependence of preservation on

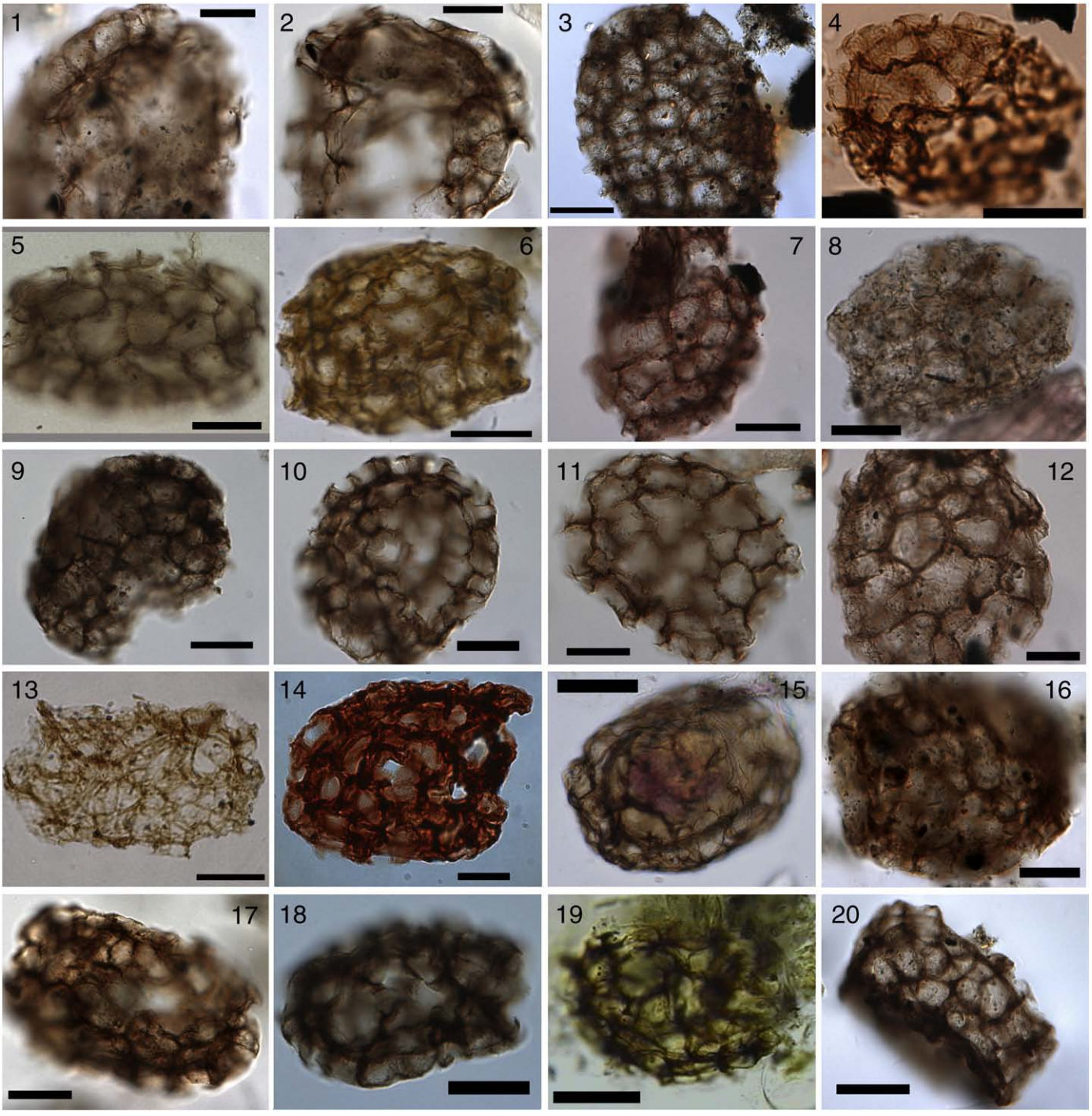


Plate II.

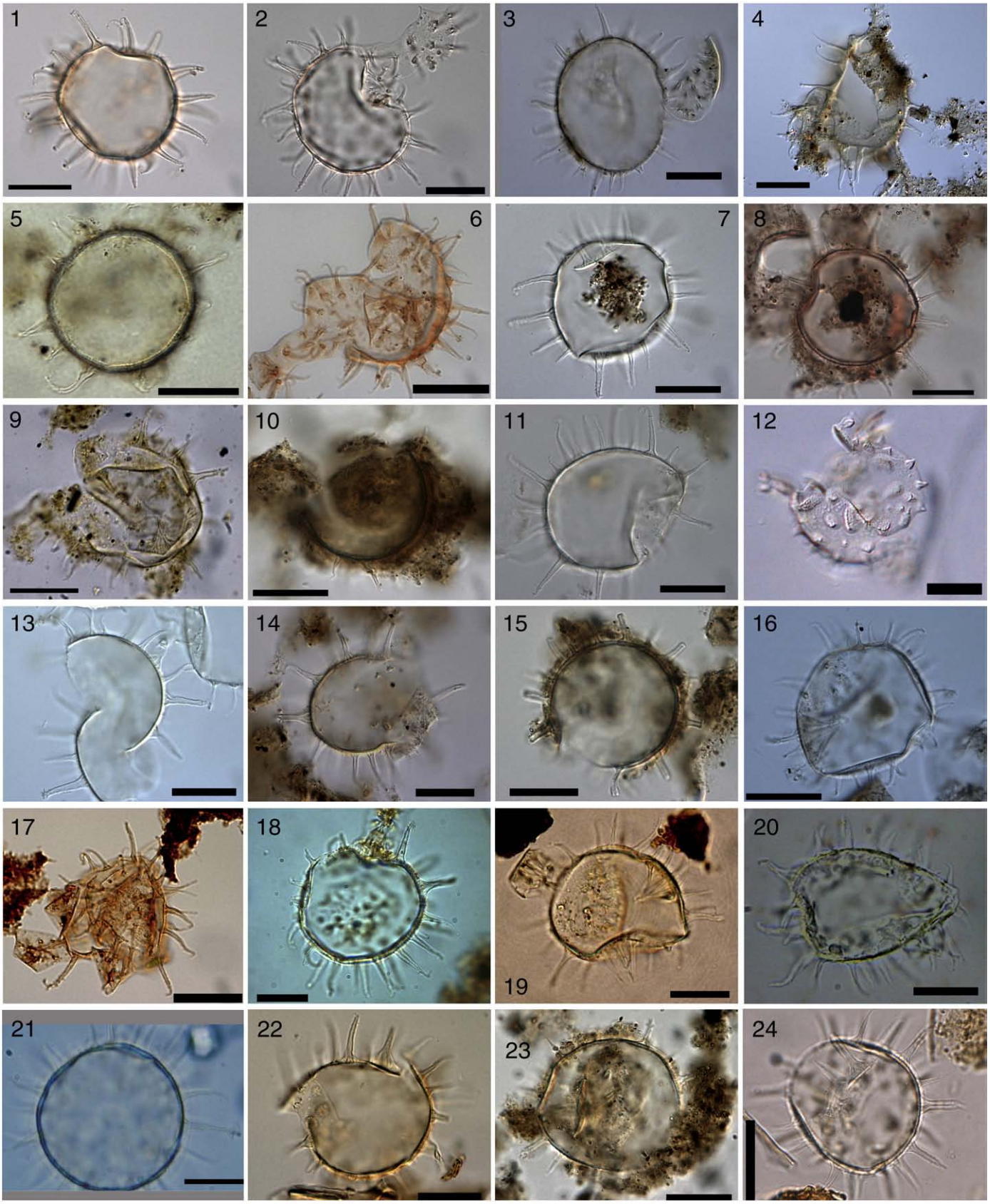


Plate III (*see caption on page 242*).

methodology, it is necessary to group the present species according to their resistance to degradation. It is assumed that both mechanical and chemical degradation have similar effects on an assemblage. The grouping proposed here is similar to the grouping described by Zonneveld et al. (2001). Cysts not referred to by these authors were added to a particular group based on the assumption that comparable morphology (e.g. wall thickness, resistance of structures against folding) is indicative of similar resistance to decay.

Extremely sensitive cysts: cysts of *Alexandrium* spp., *Dalella chathamense,* cysts of *Gymnodinium* spp., *Lejeunecysta* spp., *Polykrikos* spp., round brown cysts (RBC), *Selenopemphix* spp., spiny brown cysts (SBC), *Stelladinium* spp., *Tuberculodinium vancampoae* and *Xandarodinium xanthum.*

Moderately sensitive cysts: *Lingulodinium machaerophorum*, *Operculodinium* spp., *Pyxidinopsis reticulata*, *Quinquecuspis concreta, Spiniferites* spp., *Trinovantedinium applanatum* and *Votadinium* spp.

Resistant cysts: *Ataxiodinium choane*, *Impagidinium* spp., *Nematosphaeropsis labyrinthus*, *Operculodinium israelianum*, *Pentapharsodinium dalei*, *Polysphaeridium zoharyi* and *Bitectatodinium* spp.

It is evident from the dataset that some species were not recorded by some observers. One obvious example is *Dubridinium* spp., which was often counted by some laboratories as RBC or not counted at all. To partly reduce this observer bias, we decided to group species into genera or larger groups (Appendix A). Averages of relative abundances were only calculated when at least 300 dinoflagellate cysts were counted. The counts from oxidized samples (laboratory 22b) were also excluded, since all heterotrophic cysts were destroyed. The average results of the four samples are shown in Table 2. Representative cysts from the four samples are shown in Plates I–IV.

3.2. Absolute abundances of dinoflagellate cysts

The cyst concentration (absolute abundance) in the North Sea sample ranges from 570 to 3304 cysts/g, excluding the outliers: laboratory 1a produced a very high number (8342 cysts/g) and laboratory 22b a very low number (278 cysts/g). The average is 1516 cysts/g with a standard deviation of 698 cysts/g (coefficient of variation, $V=46\%$). The average coefficient of variation from the confidence limits of Maher (1981) is 20%. The volumetric method was not used for the North Sea sample (Table 3).

The cyst concentration (absolute abundance) in the Celtic Sea sample ranges from 1240 to 5284 cysts/g, excluding the outliers: laboratories 14 and 1a produced high numbers of 75,633 and 10,961 cysts/g respectively, while laboratory 20a, 2 and 20b give respectively low values of 1053, 731 and 501 cysts/g. The average is 2583 cysts/g, with a standard deviation of 1342 cysts/g ($V=52\%$). The average coefficient of variation from the confidence limits of Maher (1981) is 25%. Results obtained by the volumetric method give estimates that are much lower than with the marker grain method. For the Celtic Sea these values (1160 cysts/g (laboratory 6) and 1167 cysts/g (laboratory 8)) are even below the lowest value obtained by the marker grain method (Table 3).

The cyst concentration (absolute abundance) in the NW Africa sample ranges from 4606 to 38,357 cysts/g, excluding the outliers: laboratories 11, 1a and 14 produced very high numbers (168,899, 167,651 and 129,236 cysts/g, respectively). The average is 19,441 cysts/g, with a standard deviation of 9148 cysts/g ($V=47\%$). The average coefficient of variation from the confidence limits of Maher (1981) is 23%. As before, the volumetric method gave lower estimates but within the range of the marker grain method (11,600 cysts/g (laboratory 6) and 9992 cysts/g (laboratory 8)) (Table 3).

The cyst concentration (absolute abundance) in the Benguela sample ranges from 30,130 to 298,972 cysts/g, excluding the outliers: Laboratory 1c produced a high number of 1,455,988 cysts/g, while laboratories 20b and 8 give values as low as 18,472 and 15,910 cysts/g, respectively. The average is 144,299 cysts/g with a standard deviation of 84,159 cysts/g ($V=58\%$). The average coefficient of variation from the confidence limits of Maher (1981) is 21%. The volumetric method used by laboratory 6 yields 53,200 cysts/g (within the range above) and 8492 cysts/g by laboratory 8. The volumetric estimate by laboratory 8 is considered to be an underestimation caused by the destruction of fragile cysts by sonication (see Discussion); (Table 3).

3.3. Reworked dinoflagellate cysts

About 7% of the recorded dinoflagellate cysts in the North Sea sample were reworked. The pre-Quaternary cysts recorded in the North Sea sample were *Wetzeliella* spp. (dominant), *Glaphyrocysta* spp., *Cordosphaeridium* spp., cf. *Oligosphaeridium* spp. and cf. *Cribroperidinium* spp. In terms of absolute abundances, reworking shows the same trends as *in situ* dinoflagellate cyst absolute abundances. Very high absolute abundances were recorded in the sample oxidized by laboratory 22b. This indicates that the robust pre-Quaternary cysts are more resistant to oxidation. Reworking is very low (less than 1%) in the samples from the Celtic Sea, NW Africa and Benguela.

3.4. Other palynomorphs

Chlorophycean palynomorphs such as *Cymatiosphaera* sp. (not present in Celtic Sea), *Pediastrum* sp., *Pterospermella* sp. (not present in Benguela), *Tasmanites* sp., *Botryococcus* sp. (not present in Benguela), *Mougeotia* sp. (only North Sea), *Concentricystes circulus* (only NW Africa), *Gelasinicysta* sp. indet. (only NW Africa) are recorded in low numbers in all samples, except the North Sea sample.

Faunal remains such as microforaminiferal linings, scolecodonts, tintinnids, planktonic crustacean eggs and invertebrate mandibles were encountered in almost every sample. Planktonic crustacean eggs are very abundant in the North Sea sample.

Pollen and spores are abundant in the North Sea sample. The assemblage is dominated by pollen (90%). Non-bisaccate pollen include *Quercus*, *Corylus, Betula, Alnus*, pollen of Poaceae, Cyperaceae and Chenopodiaceae, whereas bisaccate pollen comprise mainly *Pinus* and *Picea.* Some *Cedrus* pollen is recorded. Reworked pollen and spores are present in low numbers.

The Celtic Sea sample is dominated by pollen (94%). Non-bisaccate pollen comprises mainly pollen of Poaceae, *Quercus*, pollen of Ericaceae and Chenopodiaceae. Bisaccate pollen is mainly *Pinus* pollen. Reworked pollen and spores are very rare.

The sample from NW Africa is also dominated by pollen (95%). Non-bisaccate pollen comprise mainly pollen of Poaceae, *Quercus*, pollen of Ericaceae and pollen of Chenopodiaceae. The bisaccate pollen are mainly *Pinus* pollen. Reworked pollen and spores are very rare.

The Benguela assemblage is dominated by pollen (99%). Non-bisaccate pollen includes mainly pollen of Poaceae, Asteraceae and Caryophyllaceae. Bisaccate pollen is mainly *Pinus* pollen. No reworked pollen and spores were recorded.

Hyphae and fruiting bodies were counted as fungal remains in order to check whether the samples were infected by fungi. No samples showed significant abundances.

The recorded incertae sedis include *Cyclopsiella*, *Halodinium* sp., *Hexasterias problematica* (not present in Northwest Africa), *Micrhystridium* sp. (Celtic Sea and Benguela), *Palaeostomocystis subtilitheca* (North Sea and Celtic Sea), *Radiosperma corbiferum* (Celtic Sea and Benguela) and *Sigmopollis* sp. (NW Africa). These were more abundant in both North Sea and Celtic Sea samples.

Other organisms occurring are the organic linings of calcareous dinoflagellate cysts, thecamoebians (North Sea, Celtic Sea), chrysomonad cysts (North Sea, Celtic Sea) and diatoms. Diatoms can still be present when low concentrations of HF are used, possibly combined

with heavy liquid separation, which enhances the abundance of diatoms with low densities (laboratories 1c, 9 and 17). Laboratory 20b has good recovery of diatoms, since the samples are not treated with HF.

4. Discussion

4.1. Is a 300 or 400 dinoflagellate cyst count sufficient to reach reliable diversities and absolute abundances?

There is no general agreement on the number of cysts which should be counted to obtain reliable data for diversity and absolute abundance studies. Most palynologists usually count 300 cysts per sample, which can provide up to 98% confidence (Germerad et al., 1968). To check whether it is necessary to count 300 or 400 dinoflagellate cysts, results from counting 300 cysts, plus an additional 100 cysts are compared using absolute abundances, species diversity and the Shannon–Wiener Index for all samples (Table 3). The comparison shows that the disparities in the results are insignificant: averages of absolute abundances, species richness and the Shannon–Wiener Index show limited changes compared to the associated standard deviations. The statistical test of Maher (1981) indicates that all absolute abundances derived from the 300 dinoflagellate cyst count statistically produce the same concentration as from the 400 dinoflagellate cyst count. It can thus be concluded that a 300 dinoflagellate cyst count is sufficient for generating reliable diversities and absolute abundance data in Quaternary studies.

4.2. Reproducibility of relative abundances

The standard deviations of the relative abundances observed in the grouping based on cyst preservation are always lower than 11.2%. These relatively small standard deviations suggest that changes in the relative abundance counts are caused by observer bias rather than by differences in methodology. Indeed, the highest standard deviations in the taxonomical groupings are with the taxa RBC, SBC and *Lejeunecysta* s.l. and since it can be assumed that the potential for preservation of these taxa is similar, it is likely that the disparities in the counts are the result of observer bias. The high standard deviation for RBC is probably caused by the high numbers of the morphologically similar *Dubridinium* spp. and the unfamiliarity of many observers with *Dubridinium* spp. Furthermore, an unambiguous definition of a round brown cyst is still lacking. The same is true for the spiny brown cysts, and several poorly defined species fall within this group. All other standard deviations are lower than 10%, which we consider an acceptable range for completely independent dinoflagellate cyst counts. Another possible reason for observer bias could be related to the use of different illumination techniques for routine counting of dinoflagellate cysts. Comparison of the use of phase contrast to interference contrast illumination to count dinoflagellate cysts on the same slides by laboratory 15 revealed that phase contrast emphasizes the transparent cysts (*Spiniferites* s.l., *Operculodinium* s.l., *Nematosphaeropsis labyrinthus*, etc.), whilst interference contrast emphasizes the brown heterotrophic cysts (RBC, SBC, etc.). Despite the observer bias, there is no doubt that dinoflagellate cyst relative abundance counts by one single observer are repeatable.

4.3. Explanation of outliers in absolute abundances

The higher numbers can each be explained by examining specific methodologies employed by particular labs. Labs 1a and 1c lost an excessive amount of *Lycopodium* spores due to the use of sieving at 20 μm as shown by Lignum et al. (2008). Labs 11 and 14 experienced problems with settling after centrifugation and were not confident that the final residues were suitable for quantitative analysis.

The lower numbers by laboratory 22b are due to the use of oxidation, which causes preferential destruction of dinoflagellate cysts. Due to the low amounts of material used in the exercise, the maceration tank and washing machine method (laboratories 20a and 20b) did not function optimally and yielded atypical results that should not be regarded as representative. This might be due to cysts getting attached to the large filter cloth (25×25 cm) used in this technique (see Discussion, assumption 8). Furthermore, one of the samples from NW Africa (laboratory 20b) was separated at specific gravities of 1.8, 2.0 and 2.3 g/ml. At the specific gravities of 1.8 and 2.3 g/ml, there were almost no dinoflagellate cysts in the slides, whereas ten times more dinocysts were noted at the specific gravity of 2.0 g/ml. Further investigation is needed to evaluate the effect of heavy liquid separation at different specific gravities.

For laboratory 8, the use of a sonic oscillator resulted in destruction of sensitive cysts, again yielding lower numbers.

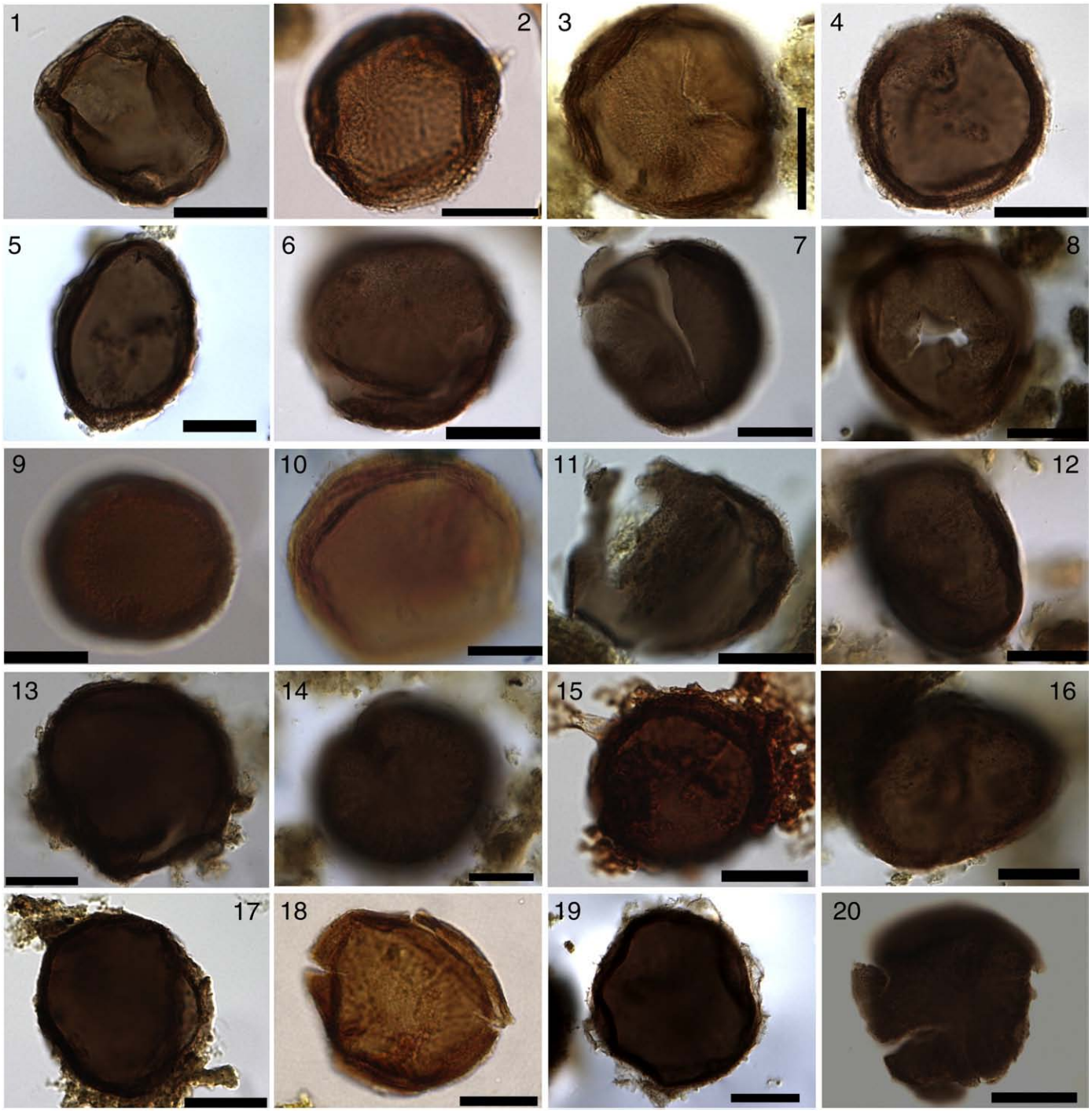
4.4. Reproducibility and accuracy of absolute abundances, excluding the outliers

Total cyst count is less dependent on taxonomical expertise, and thus probably less influenced by observer bias. The different laboratories participating in the current inter-calibration exercise used different processing techniques (see Supplementary data). The

Plate IV. *Dubridinium* spp. extracted from the Benguela sample using different methodologies, sorted from high (upper left corner) to low absolute abundances (lower right corner).

1. Lab 1c.
2. Lab 3.
3. Lab 19.
4. Lab 11.
5. Lab 13.
6. Lab 1a.
7. Lab 21a.
8. Lab 21b.
9. Lab 6.
10. Lab 16.
11. Lab 18a.
12. Lab 18b.
13. Lab 1b.
14. Lab 23.
15. Lab 10b.
16. Lab 17.
17. Lab 10a.
18. Lab 5.
19. Lab 2.
20. Lab 8. Destructive ultrasonication. All scale bars are 20 μm.

reproducibility of estimates of absolute cyst abundances, as expressed as coefficient of variation in Table 2, shows that there are differences among the 23 laboratories: the coefficients of variation are relatively large (46–58%) and nearly twice as high as the coefficients of variations (20–25%) which are calculated from Maher (1981). Our results suggest that the determination of absolute abundances is mainly dependent on processing methodology. In this light the accuracy also needs to be considered: a better understanding of what is causing the variation can only be achieved when correct absolute abundances of dinoflagellate cysts have been determined. To estimate whether the absolute abundances give an accurate picture of the true absolute abundances of the dinoflagellate cysts, results from the marker-grain method are compared with independent methods. When compared to the volumetric method, absolute abundances calculated using the marker-grain method, are 44–63% higher (Table 2). In a similar study, de Vernal et al. (1987), noted systematically higher concentrations from the marker-grain method compared to the results from the volumetric method, and they suggested that significant losses of *Lycopodium* spores (close to 33% on the average) took place during laboratory procedures. On the other hand, in a study on Paleogene sediments, Heilmann-Clausen (1985), found marker-grain estimates varying between 70% and 129% of volumetric estimates and on average 2% lower concentration was calculated from the marker-grain method. Our study confirms the



observation of de Vernal et al. (1987), and even shows larger deviations. It should also be noted, that counts from strew slides made from unprocessed samples show much lower abundances than the average absolute abundances from the marker grain method. From these observations, it can be concluded that with most preparation techniques there are significant losses of *Lycopodium* spores, and this is most probably the reason for higher the absolute abundances using the marker-grain method. Furthermore, there was no evidence of significant loss of dinoflagellate cysts during the laboratory preparations, except when oxidation or very long or destructive sonication was used (see below). Thus, in order to understand what causes the differences in absolute abundances, one needs to consider underlying assumptions. Ten assumptions need to be considered.

(1) Drying samples does not cause decay.

Although drying is often done in palynological preparation, it should be avoided in organic rich sediments, where drying causes formation of selenite (gypsum, $CaSO_4 \cdot 2H_2O$), by reaction of calcium carbonate with sulphuric acid, usually derived from pyrite decay. The formation of sulphuric acid significantly affects extremely sensitive dinoflagellate cysts. In this case, to calculate the weight of the samples, wet volumes should be used, corrected with dry bulk densities. In our samples, gypsum crystals were not observed. The homogenized samples were oven dried before subdivision into smaller batches and dispatching to individual laboratories. This was done to avoid differential drying. However, not all laboratories processed the samples exactly at the same time. Samples were dispatched in March 2007, and were processed within the following year. The possibility exists that samples that were processed at a later stage dried out more. Clustering of amorphous organic matter around the cysts seems to occur in more dried out samples (most obvious around *Lingulodinium machaerophorum* specimens in Plate III), but there were no clear signs that this process caused changes in the assemblage. This assumption is thus acceptable.

(2) Samples are homogenous.

It needed testing if samples processed in a similar manner yielded reproducible results. All samples were processed twice by laboratory 21 (a and b) with the only difference in preparation the addition of some soap during sieving (Table 4). Following the test by Maher (1981), for every studied sample, the microfossil concentration in the quasi-replicas is the same. It can thus be concluded that the samples are well-mixed and are homogenous. Furthermore, there are few differences between both samples in terms of relative abundances. This assumption is thus acceptable.

(3) A single *Lycopodium* tablet from batch 483216 contains 18,583 ± 1708 spores.

This reference is given by the supplier (Lund University), and these numbers were calibrated using a Coulter counter. Lignum et al. (2008) also used a Coulter counter for verification and obtained 16,971 ± 1251 *Lycopodium* spores. We dissolved one tablet in distilled water and sieving on a 0.25 µm Millipore filter. The filter was cut into two pieces, mounted on a slide and counted under a transmitted light microscope. On this filter, 16,993 *Lycopodium spores* were counted, which falls within the range proposed by the supplier and Lignum et al. (2008). A similar exercise has been done for another batch by Stabell and Henningsmoen (1981) which found similar results. This assumption is thus acceptable.

(4) There is no degradation of palynomorphs caused by chemical treatment such as oxidation or acid treatments by HF and HCl.

Since *Lycopodium* spores are acetolysed during the manufacturing process, they can withstand acetolysis. Effects of chemicals on *Lycopodium* show that only colour changes are caused by acetolysis or HCl treatment (Sengupta, 1975). On the other hand, it has been shown that acetolysis or oxidation selectively destroys the cysts of the Polykrikaceae and Protoperidiniaceae (Reid, 1977; Marret, 1993). KOH treatment causes destruction of the Protoperidiniaceae after 5 min (de Vernal et al., 1996, and Mertens, pers. observations) and causes swelling of the palynomorphs. Likewise, methods using H_2O_2 (Riding et al., 2007) result in the destruction of protoperidiniacean cysts (Riding, pers. comm., Hopkins and McCarthy, 2002; Mertens, pers. obs.). This has also been demonstrated for Late Cretaceous peridinioid dinoflagellate cysts (Schrank, 1988). Oxidation with Schulze's solution by laboratory 22b resulted in the near complete destruction of the RBC, SBC and other heterotrophs in all samples, and led to the relative enrichment of resistant pollen and reworked non-peridinioid dinoflagellate cysts. Cold HF and HCl have never been reported to destroy dinoflagellate cysts. However, hot rinses with HCl after the HF treatment were particularly harmful to recent peridinoid cysts (Dale, 1976). Palynomorphs treated with warm HF clearly showed traces of deterioration: destruction of delicate structures with fragmentation along sutures and changes in wall texture with a thickening of the robust structures (Plate I, 11, 16, Plate III, 6). It can be concluded that this assumption is acceptable when chemical degradation is minimized by using only cold hydrochloric and hydrofluoric acid.

(5) Sonication causes no mechanical degradation of the pollen and spores or dinoflagellate cysts.

The extensive use of ultrasound will not harm any dinoflagellate cysts according to Funkhouser and Evitt (1959), however, other authors report differential damage (e.g. Hodgkinson, 1991). This has not yet been checked in a quantitative manner for dinoflagellate cysts. The use of a sonic oscillator, although dependent on frequency (Marceau, 1969) is extremely damaging: the sonication by laboratory 8 resulted in the destruction of RBC and SBC in the Benguela sample (Plate IV, 20). Laboratory 18a used an ultrasonic bath for 30 min, and this resulted in extensive damage to the cysts. Many cysts were fragmented, often with broken or even lost spines and were often clustered (Plate I, 17, Plate III, 13, Plate IV, 11). In addition microforaminiferal linings were often fragmented. This assumption is thus acceptable when an ultrasonic bath is not used for too long. A limit of 60 s is proposed.

(6) Centrifugation causes no mechanical degradation of the palynomorphs.

No visible signs were noted that this technique causes degradation of the cysts. This assumption is thus acceptable.

(7) Sieving causes no loss of palynomorphs.

Lignum et al. (2008) demonstrated that sieving should be done with a sieve mesh width smaller than 15 µm. Our results confirm this observation. Laboratories using nylon sieve with widths of 20 µm (laboratories 1a and 1c) showed extremely high absolute abundances. This suggests that significant losses of *Lycopodium* spores occurred during the sieving process — even larger than the 20% that is proposed by Lignum et al. (2008). No significant loss of cysts was documented in this study. It is possible that cysts of *Pentapharsodinium dalei* pass through 20 µm sieves, this species was present in such low abundances in the studied samples to significantly affect relative or absolute abundances. This assumption is thus acceptable when mesh sizes smaller than 15 µm are used.

(8) Decantation causes no loss of palynomorphs.

An experiment was done to determine how many *Lycopodium* spores were lost during decanting and sieving. One gram of the NW

Table 3
Comparison between the marker-grain method and the volumetric method.

Method	Variable/sample	North Sea	Celtic Sea	NW Africa	Benguela
Marker grain method	Average (cysts/g)	1516	2583	19,441	144,299
	St dev (cysts/g)	698	1342	9148	84,159
	Coefficient of variation (%)	46	52	47	58
	Coefficient of variation (%) Maher (1981)	20	25	23	21
Volumetric method	Average (cysts/g)		1163	10,796	53,200
	St dev (cysts/g)		5	1137	0
	Coefficient of variation (%)		0	11	0
Difference	Cysts/g	–	1420	8645	91,099
	%		55	44	63

Table 5
The results of the counts of samples processed and counted by Lab 21, processed with one processing technique. According to the statistical test by Maher (1981), the results are reproducible.

Lab number	Variable/sample	North Sea	Celtic Sea	NW Africa	Benguela
21a	Dinoflagellate cysts/g	1547	2581	27,851	172,078
	95% confidence limits (Maher, 1981)	1265–1885	2092–3327	21,612–32,060	138,365–206,955
21b	Dinoflagellate cysts/g	1447	2723	24,929	170,888
	95% confidence limits (Maher, 1981)	1166–1785	2117–3354	19,294–28,216	135,585–200,884

Africa sample together with one *Lycopodium* tablet, was processed with a HCl/HF/HCl cycle, followed by sieving on a nylon mesh of 10 µm. After every decantation, the decanted fluid was filtered through a 0.25 µm Millipore filter. What remained on the filter was counted under a transmitted light microscope. Only *Lycopodium* spores were left on the filters, as well as some amorphous organic matter (Table 5). The number of spores will be dependent of the size of the filter used. Apparently 24% of the *Lycopodium* spores were lost during decanting. This is not surprising, since it is well-known that *Lycopodium* spores float (e.g. Salter et al., 2002). An extra 1.3% was left on the filter and 1% got stuck to handling material (e.g. spatula, tube). In the slides only 43.4% of the *Lycopodium* spores were found. An additional 30.2% spores were unaccounted for, and could have been lost during sieving and/or could have been obscured by other material in the slides to some extent. Because we did not expect any significant losses to occur during sieving, we did not capture sieved material during this experiment. However, we tested sieving a complete *Lycopodium* tablet on 10 µm and capture on a 0.25 µm sieve. We found losses to be 0.79% when gently pouring the dissolved tablet over the sieve and subsequent washing, 0.97% when using a hand pump to facilitate sieving and 2.01% when using a pipette tip. Lignum et al. (2006) recorded losses up to $5.8 \pm 1.2\%$ for 15 µm meshes. It can thus be assumed that only a small part of the missing spores were pushed through the 10 µm nylon sieve. Presumably, spores are often concealed by being obscured by other material, and this plays a more significant role in explaining the missing amount of spores. Also, it is possible that due to the texture of the exines of *Lycopodium* spores, the spores get more easily caught in the sieves than smoother palynomorphs. However, this loss can be easily checked by the observer. This assumption is thus not acceptable.

(9) Pre-sieving causes no losses.

It is unclear to what extent presieving causes loss of *Lycopodium* spores, although it is evident that it should be avoided in samples from high productivity areas, where high production of amorphous organic matter forms large clusters in the sediment, which can be discarded with the large fraction. However; it can be easily checked whether *Lycopodium* spores were lost.

(10) Heavy liquid separation causes no loss of *Lycopodium* spores.

It has been noted that density separation with heavy liquids can cause incorporation of mineral particles modifying the density of the heavy liquid (de Vernal et al., 1996). Litwin and Traverse (1989) recommend pyrite to be removed prior to density separation. The results of this study do not show any obvious difficulties with this processing step, although for clarity further study is suggested.

From these considerations it can be concluded that a significant amount of *Lycopodium* spores are lost, mainly during decanting and sieving. There is little evidence that there is loss of dinoflagellate cysts during these manipulations (Table 6).

5. Conclusions and recommendations

(1) This study was designed as a comparative one, where the degree of variability in preparations could be objectively assessed. The laboratories concerned agreed to take part on the basis that the results would be presented anonymously, in order to ensure maximum participation. The point of this work was to carefully study the techniques used and to encourage best practice in the future. This initial work presents a firm basis for more methodological research.
(2) The exercise demonstrated that relative abundances are reproducible, but underlined the urgent need for taxonomic intercalibration.
(3) The study also shows that counting 300 dinoflagellate cysts is sufficient both in terms of diversity and absolute abundances.

Table 4
Comparison between the average results after counting 300 dinoflagellate cysts, and counting 400 dinoflagellate cysts.

Variable/sample	North Sea 300 cysts	North Sea 400 cysts	Celtic Sea 300 cysts	Celtic Sea 400 cysts	NW Africa 300 cysts	NW Africa 400 cysts	Benguela 300 cysts	Benguela 400 cysts
Average (cysts/g)	1539	1546	2792	2670	33,798	33,684	141,825	142,612
St dev	767	711	1474	1236	43,286	42,193	87,324	88,779
Coefficient of variation (%)	50	46	53	46	128	125	62	62
Species richness	22.00	22.85	24.26	25.26	14.75	16.50	19.13	20.22
St dev	4.67	4.79	5.61	6.02	3.64	4.12	4.94	5.27
Shannon–Wiener index	2.25	2.25	2.29	2.29	0.70	0.72	1.94	1.92
St dev	0.41	0.41	0.30	0.32	0.22	0.23	0.35	0.33

(4) Absolute abundance calculations of dinoflagellate cysts are dependent on processing methodology, since *Lycopodium* spores are being lost during different processing steps.
(5) It is possible that some of the laboratories consistently over- or underestimate concentrations. The addressed problems in methodology might partly explain these outliers. Future work should elucidate possible corrections by detailed investigation of every different processing step.
(6) At the current state of affairs, there are three possible choices the Quaternary worker can make to calculate reproducible absolute abundances:
   1. Standardize methodology for the extraction of dinoflagellate cysts.
      Since samples can be reproducible when one fixed methodology is followed (see Section 4.3), a standard methodology is suggested (Fig. 1). We consider that there are critical steps that must be avoided in this standard method when preparing samples for dinoflagellate cyst work: the use of oxidation, KOH, warm acids, acetolysis, mesh sizes larger than 15 µm, decanting (substituted by sieving) and sonication longer than 1 min. During sieving, care should be taken to avoid *Lycopodium* spores being forced through the sieve. A certain degree of freedom is allowed in the number of HCl and HF cycles, length of ultrasonication (0–60 s), duration of sieving and sieve mesh size (6–14 µm), Care should be taken to neutralize HF by diluting at least ten times before sieving. Further studies are required to fine-tune the method by focusing on designated issues.
   2. Adding *Lycopodium* tablets at the end of processing.
      The marker grain method is based on the assumption that there is no selective loss of fossil and exotic pollen during the procedures. However, this assumption has never been checked. Our study suggests that predominantly *Lycopodium* spores are lost, and that losses of dinoflagellate cysts are negligible. Therefore the addition of *Lycopodium* tablets at the end of the preparation is suggested, thus limiting the loss of *Lycopodium* spores. However, this method is contrary to spiking with an internal standard before the start of preparation.
   3. Alternative methods.
      Alternative methods can be used, but may not yield better results. The use of microbeads was introduced by Ogden (1986), but often results in much higher abundance estimates, apparently because of difficulty in sustaining an even suspension of the particles in the stock solution: the higher specific gravity of microspheres causes them to settle three to four times more rapidly than pollen grains (McCarthy, 1992). Other marker-grain methods, such as the *Eucalyptus globulus* marker-grain method (Matthews, 1969), has also been used (e.g. de Vernal et al., 1987). However, it is not known whether these methods give more reliable results. The aliquot method gives more accurate results than the *Lycopodium* method in our study, but unfortunately not much is known about the precision of this method.

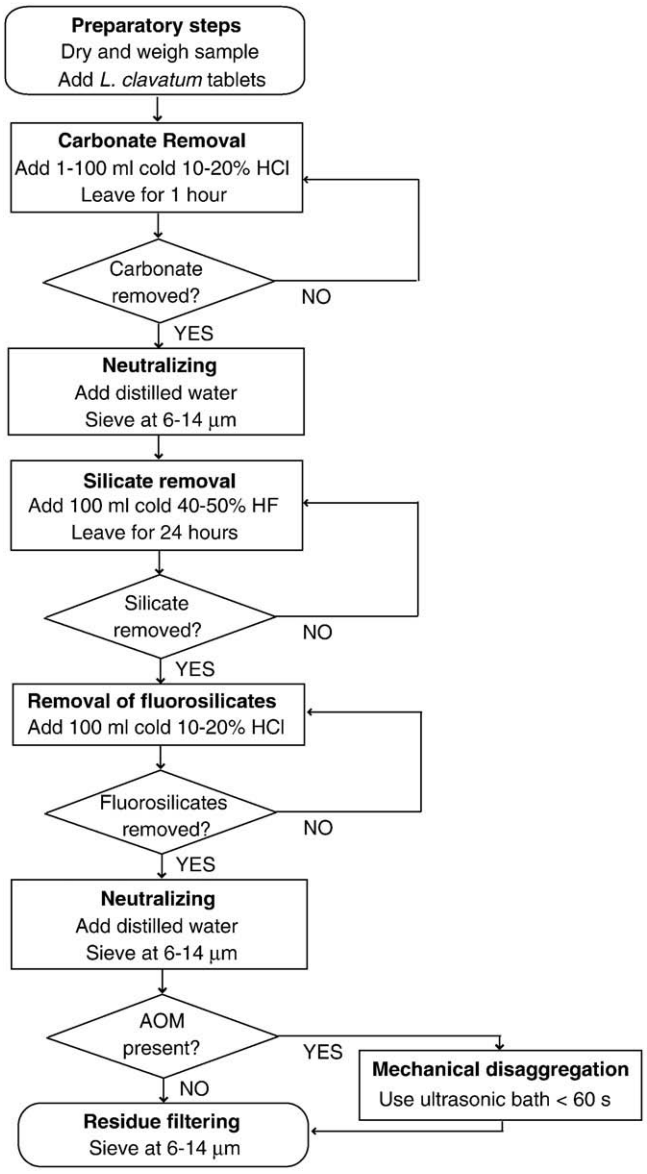


Fig. 1. Flow-chart of the proposed standardized method. AOM stands for amorphous organic matter.

Table 6
Results of an experiment to look into the effects of manipulations on loss of *Lycopodium* spores. Shown is the number of *Lycopodium* spores lost during each manipulation. It is supposed that one tablet contains 18,583 spores, so the % is calculated by dividing the number of counted spores by 18,583 spores.

	Counted *Lycopodium* spores	%
HCl treatment		
First decantation	916	4.9
Second decantation	267	1.4
Third decantation	2485	13.4
HF/HCl treatment		
First decantation	6	0.0
Second decantation	143	0.8
Third decantation	650	3.5
Left on filter (not washed off)	242	1.3
Left in tube + stuck on spatula	187	1.0
Found on slides	8067	43.4
Total	12963	69.8
Missing spores	5620	30.2

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Appendix A. Species list.

Species name	Grouped under	North Sea	Celtic Sea	NW Africa	Benguela
Achomosphaera andalousiensis Jan du Chêne 1977	*Spiniferites* s.l.	x	x	x	
Cysts of *Alexandrium affine* (Ioue and Fukuyo 1985) Balech 1985	Cyst of *Alexandrium* spp.		x		x
Cysts of *Alexandrium tamarense* (Lebour 1925) Balech 1985	Cyst of *Alexandrium* spp.	x	x		
Ataxiodinium choane Reid 1974	*Ataxiodinium choane*	x	x	x	
Bitectatodinium spongium Zonneveld 1997	*Bitectatodinium* spp.		x	x	x
Bitectatodinium tepikiense Wilson 1973	*Bitectatodinium* spp.	x	x	x	x
Tectatodinium pellitum Wall, 1967 emend. Head 1994	*Tectatodinium* spp.				x
cf. Tectatodinium pellitum Wall, 1967 emend. Head 1994	*Tectatodinium* spp.	x			
Brigantedinium cariacoense (Wall 1967) Lentin and Williams 1993	Round Brown Cyst	x	x	x	x
Brigantedinium majusculum Reid 1977 ex Lentin and Williams 1993	Round Brown Cyst	x	x		
Brigantedinium simplex Wall 1965 ex Lentin and Williams 1993	Round Brown Cyst	x	x	x	x
Cyst of *Protoperidinium americanum* (Gran and Braarud 1935) Balech 1974	Round Brown Cyst	x	x	x	x
Dalella chathamense McMinn and Sun 1994	*Dalella chathamense*				x
Diplopelta? symmetrica Pavillard 1993 (Dale et al., 1993)	Spiny Brown Cysts			x	
Dubridinium ulsterum Reid 1977	Round Brown Cyst	x		x	x
Dubridinium caperatum Reid 1977	Round Brown Cyst	x	x	x	x
Echinidinium aculeatum Zonneveld 1997	Spiny Brown Cysts	x	x	x	x
Echinidinium bispiniformum Zonneveld 1997	Spiny Brown Cysts			x	x
Echinidinium delicatum Zonneveld 1997	Spiny Brown Cysts	x	x	x	x
Echinidinium granulatum Zonneveld 1997	Spiny Brown Cysts	x	x	x	x
Echinidinium transparantum Zonneveld 1997	Spiny Brown Cysts	x		x	x
Echinidinium cf. *transparantum* Zonneveld 1997	Spiny Brown Cysts	x	x		x
Cyst of *Gymnodinium catenatum* Graham 1943	Cyst of *Gymnodinium* spp.	x	x	x	x
Cyst of *Gymnodinium microreticulatum* Bolch et al., 1999	Cyst of *Gymnodinium* spp.	x	x		
Cyst of *Gymnodinium nolleri* Ellegaard and Moestrup 1999	Cyst of *Gymnodinium* spp.	x	x	x	x
Impagidinium aculeatum (Wall 1967) Lentin and Williams 1981	*Impagidinium* spp.		x		
Impagidinium pallidum Bujak 1984	*Impagidinium* spp.		x		
Impagidinium paradoxum (Wall 1967) Stover and Evitt 1978	*Impagidinium* spp.	x	x		x
Impagidinium patulum (Wall 1967) Stover and Evitt 1978	*Impagidinium* spp.	x	x	x	
Impagidinium sphaericum (Wall 1967) Lentin and Williams 1981	*Impagidinium* spp.	x	x		x
Impagidinium strialatum (Wall 1967) Stover and Evitt 1978	*Impagidinium* spp.				x
Impagidinium velorum Bujak 1984	*Impagidinium* spp.	x		x	
Islandinium? cezare de Vernal et al., 1989 ex de Vernal in Rochon et al., 1999	Spiny Brown Cysts	x			
Islandinium minutum Harland and Reid in Harland et al., 1980	Spiny Brown Cysts	x	x	x	x
Leipokatium invisitatum Bradford 1975	*Lejeunecysta* s.l.		x		
Lejeunecysta diversiforma (Bradford 1977) Artzner and Dörhöfer 1978	*Lejeunecysta* s.l.				x
Lejeunecysta marieae Harland in Harland et al., 1991 ex Lentin and Williams 1993	*Lejeunecysta* s.l.	x			
Lejeunecysta oliva (Reid 1977) Turon and Londeix 1988	*Lejeunecysta* s.l.	x	x	x	x
Lejeunecysta paratenella (Benedek 1972) Zonneveld and Marret xxx	*Lejeunecysta* s.l.	x	x		x
Lejeunecysta sabrina (Reid 1977) Bujak 1984	*Lejeunecysta* s.l.	x	x	x	x
Lingulodinium machaerophorum (Deflandre and Cookson 1955) Wall 1967	*Lingulodinium machaerophorum*	x	x	x	x
Nematosphaeropsis labyrinthus (Ostenfeld 1903) Reid 1974	*Nematosphaeropsis labyrinthus*	x	x	x	x
Operculodinium centrocarpum sensu Wall and Dale (1966)	*Operculodinium* s.l.	x	x	x	x
Operculodinium israelianum (Rossignol 1962) Wall 1967	*Operculodinium israelianum*	x	x	x	x
Operculodinium janduchenei Head et al., 1989	*Operculodinium* s.l.	x	x	x	x
Operculodinium sp. II? Marret, 1994	*Operculodinium* s.l.				x
Operculodinium sp. A of Vink (2000)	*Operculodinium* s.l.			x	
Cyst of *Pentapharsodinium dalei* Indelicato and Loeblich III 1986	Cyst of *Pentapharsodinium dalei*	x	x	x	x
Polykrikos kofoidii Chatton 1914	*Polykrikos* spp.	x	x	x	x
Polykrikos schwartzii Bütschli 1873	*Polykrikos* spp.	x	x	x	x
Polysphaeridium zoharyi (Rossignol 1962) Bujak et al., 1980	*Polysphaeridium zoharyi*	x	x	x	x
Pyxidinopsis reticulata (McMinn & Sun 1994) Marret and de Vernal 1997	*Pyxidinopsis reticulata*	x			
Quinquecuspis concreta (Reid 1977) Harland, 1977	*Quinquecuspis concreta*	x	x	x	x
Selenopemphix crenata Matsuoka and Bujak, 1988	*Selenopemphix* s.l.				x
Selenopemphix nephroides Benedek 1972; emend. Bujak in Bujak et al., 1980; emend. Benedek and Sarjeant 1981	*Selenopemphix* s.l.	x	x	x	x
Cyst of *Protoperidinium nudum* (Meunier 1919) Balech 1974	*Selenopemphix* s.l.	x	x	x	x
Selenopemphix quanta (Bradford 1975) Matsuoka 1985	*Selenopemphix* s.l.	x	x	x	
Spiniferites belerius Reid 1974	*Spiniferites* s.l.	x	x	x	x
Spiniferites bentorii (Rossignol 1964) Wall and Dale 1970	*Spiniferites* s.l.	x	x	x	x
Spiniferites bulloideus (Deflandre & Cookson 1955) Sarjeant 1970	*Spiniferites* s.l.	x	x		x
Spiniferites delicatus Reid 1974	*Spiniferites* s.l.	x	x	x	x
Spiniferites elongatus Reid 1974	*Spiniferites* s.l.	x	x		x
Spiniferites hyperacanthus (Deflandre and Cookson 1955) Cookson and Eisenack 1974	*Spiniferites* s.l.	x	x	x	x
Spiniferites lazus Reid 1974	*Spiniferites* s.l.	x	x		x
Spiniferites membranaceus (Rossignol 1964) Sarjeant 1970	*Spiniferites* s.l.	x	x	x	x
Spiniferites mirabilis (Rossignol 1964) Sarjeant 1970	*Spiniferites* s.l.	x	x	x	x
Spiniferites pachydermus Rossignol 1964	*Spiniferites* s.l.	x	x	x	
Spiniferites ramosus (Ehrenberg 1838) Loeblich and Loeblich 1966; emend. Davey and Williams 1966	*Spiniferites* s.l.	x	x	x	x
Stelladinium reidii Bradford 1975	*Stelladinium* spp.	x	x	x	
Stelladinium stellatum (Wall and Dale 1968) Reid 1977	*Stelladinium* spp.	x	x	x	x
Trinovantedinium applanatum (Bradford 1977) Bujak and Davies 1983	*Trinovantedinium applanatum*	x	x	x	x

(continued on next page)

Appendix A (*continued*)

Species name	Grouped under	North Sea	Celtic Sea	NW Africa	Benguela
Tuberculodinium vancampoae (Rossignol 1962) Wall 1967	*Tuberculodinium vancampoae*	x		x	x
Votadinium calvum Reid 1977	*Votadinium* spp.	x	x	x	x
Votadinium spinosum Reid 1977	*Votadinium* spp.	x	x		x
Xandarodinium xanthum Reid 1977	*Xandarodinium xanthum*	x	x	x	x

Appendix B. Error calculation according to Stockmarr (1971)

According to Stockmarr (1971) total error is $e = \sqrt{e_1^2 + e_2^2 + e_3^2}$ where

$e_1 =$ error on number of spores in marker tablets

$e_2 = \frac{\sqrt{\text{cysts counted}}}{\text{cysts counted}} =$ error on dinoflagellate cysts counted

$e_3 = \frac{\sqrt{\text{spores counted}}}{\text{spores counted}} =$ error on the number of spores counted

Appendix C. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.revpalbo.2009.05.004.

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