

FORAMINIFERA (Prof J Murray & Dr EJ Rohling)

- SAMPLING PLANKTONIC FORAMINIFERA

Living planktonic foraminifera

Living planktonic foraminiferal assemblages may be sampled using a variety of plankton-nets. Because the various planktonic foraminiferal species occupy a range of preferred depth habitats in the surface waters of the world ocean, it is best to sample discrete depth-intervals using a so-called multi-net. Planktonic foraminiferal mostly live in the upper 200 meters of the water column, although some species (notable *Globorotalia truncatulinoides*) may live down to 600 m or more. The numbers are usually expressed as numbers per litre, or per cubic metre, of sea water. This volume is estimated from the area of the open end of the net and current and vessel speeds. In some cases, more accurate direct measurements of volume flux through the net are performed.

Fossil sediments

Sampling is performed carefully, to avoid contamination, from sediment cores or outcropping sequences. Weathered sediment needs to be avoided, and in outcrops one often has to dig to get to unweathered sediments. Samples are usually taken in a fixed volume, so that numbers per cc and (after determination of accumulation rates) also numbers per cm sq per year (= fluxes) may be determined at a later stage. Careful storage and labelling is required, and it is also good practise to record positions of samples in a notebook, relative to major lithological changes (eg. "this is the last sample immediately below the ash-layer"). This will help to avoid later confusion in matching sample positions and lithological logs.

- PROCESSING PLANKTONIC FORAMINIFERAL SAMPLES

Taking care to use a well-constrained part, or all of, the original sample (so that the relation with volume remains known through the process), load sample onto adequately labelled CLEAN container (eg., petri-dish). These containers are placed in the proper oven for either freeze-drying, or normal drying (at 50 to 70 deg. C; never at higher temperatures as loss of foraminiferal shells due to cracking may occur). Dry until sample is completely dessicated. For small samples (< 5g) 24 hours at 50 deg. C will usually suffice. Freeze drying is more expensive but quicker. Dried samples should then be accurately weighed, continuously taking care not to swap samples and containers/labels, and CLEAN instruments should be used at all times. Between samples, adequate cleaning of the balance etc. is needed. It is good practise to weight labelled petri-dishes BEFORE samples are loaded into them for drying. The dishes with the dried samples can then be used straight away for weighing, after which the appropriate weight of the petri-dish may be subtracted to leave the sample dry-weight.

After weighing, samples are transferred to adequately labelled beakers, and soaked. Because of the strong carbonate content of local water, it is best to use demineralised water for soaking. Soaking should be continued with occasional stirring, until the sample has completely disaggregated (fallen apart). The sample is now ready for sieving.

- SIEVING

A sieve series/stack of the required mesh-widths is used. Normal mesh-sizes are 600, 150, 125, and 63 microns. In the plankton lab. in SOC we perform wet sieving to these sizes in one run. Other labs wet sieve to 63 microns, and later dry-sieve the residue to coarser fractions. This is a matter of choice. Samples are poured onto the sieve stack (or the 63 microns sieve) and washed GENTLY with water. A shower-head or sufficient length of flexible hose attached to the tap helps control over the direction of the water flow, and so avoids arm-breaking maneuvering of the

sieve-stack under a fixed tap. When using a stack, and when the sample has been completely washed over the upper (coarsest) sieve, this can be simply removed from the stack, and washing over next (finer) sieve is a before. NEVER rub sample over the sieve with fingers. Preferably, the only thing touching the material should be the mesh and the water stream. The stack can be gradually disassembled as above. Finally, all that is left is the 63-microns sieve, and when washing over this one is completed, the residues collected in all sieves should be collected by washing each sieve and ensuring the sample is moved into a corner, after which it can be carefully washed into a container (which was previously adequately labelled, including mention of which sieve fraction it will contain, eg. 150-600 microns).

When washing is performed with tap-water in an area with carbonate-rich water, it would be best to wash with demineralised water. When this is not practical (as at SOC), then tap water should be used, but the sieve residues should NOT be allowed to dry out. A final thorough rinse is performed with demineralised water, prior to the residues being transferred into the appropriate containers.

The containers with wet residues are placed in an oven (NOT freeze-drier) and the water is allowed to evaporate off at 50 deg C.

The dried residues are systematically weighed on a sensitive balance, and stored in appropriate storage vials.

When fractions for study seem to contain much dirty material, then a second soaking and washing may be needed, and a little CALGON may be added to the soaking solution. Weak H₂O₂ solutions are sometimes used to deal with organic dirt, but this should be done very, very, carefully and only when absolutely essential.

- COUNTING PLANKTONIC FORAMINIFERAL SAMPLES

Equipment needed:

Statistically random micro-splitter

Gridded picking tray

Fine sable paintbrush (00 or 000)

Small pot of demineralised water

Assemblage slide (normally with a black background and divided into 32 or 64 cells)

Dilute gum solution. Very finely diluted wall-paper paste works also.

Stereoscopic binocular microscope with incident light source.

Paint the assemblage slide LIGHTLY with the gum solution and allow to dry.

Picking

The residue of the desired sieve fraction (normally 150-600 microns) is used through the microsplitter as many times as needed to bring the split-fraction down to containing about 200 planktonic foraminiferal specimens. The splitter divides each run into two equal halves. Applying the process n times, therefore gives a split equal to the $1/2^n$ fraction of the original sample. This fraction is very important in re-calculating assemblage numbers to numbers per gramme total sample dryweight and/or numbers per total sample cc and/or in terms of flux per cm sq per year. Therefore, the split-fraction needs to be very carefully noted, and splitting needs to be very carefully performed.

The final split fraction is spread carefully into a gridded picking-tray, and the planktonic foraminifera are selected and transferred to the assemblage slide (sometimes also called Chapman slide) using the brush (slightly moisturised with the demineralised water). In the assemblage slide, the species are determined and separated into assigned squares. When this is done for all specimens in the sample, they can be counted.

If the observer is familiar with all the species, then it may be adequate to just count the numbers of each in the picking-tray, and dispense with the assemblage slide. The counted split-fraction may then after counting be returned to an adequately labelled storage vial.

Data

Relative abundance - % of each species within the total counted

Absolute abundance - per g of sediment, or per cm³ of sediment, or per cm sq per year (if the accumulation rate is known).

- SAMPLING BENTHIC FORAMINIFERA

Modern sediments

For quantitative studies it is necessary to collect a sample of known area/ volume of surface sediment.

Equipment

Core tube

Thin metal plate

Plunger having the internal diameter of the core tube

Retort stand to hold the plunger pointing upwards

A short section of core tube marked for the desired sample size (0.25, 0.5, 1.0 cm)

Sample containers

Methanol

Procedure

Push the core tube into the sediment and slide the metal plate beneath it. Lift out the core tube with the plate firmly held beneath.

Slide the core over the upturned plunger.

Place the short piece of core tube on the top of the core tube with sample.

Gently push the sediment core through the core tube into the short tube to the desired thickness then slide the clean metal plate between the two.

Lift off the metal plate with the sample and carefully transfer the sample into a previously labelled container; add methanol; cap; shake vigorously.

Fossil sediments

Take a piece of a core or an unweathered sample from outcrop.

Place in a labelled plastic bag and seal.

- PROCESSING FORAMINIFERAL SAMPLES

Use a paper label with the number written in pencil (not biro or any pen with ink). Keep the label with the sample at all times.

Processing involves sieving to remove fine-grained sediment and this may be followed by concentration using a heavy liquid.

If quantitative data are required, it is necessary to determine the dry weight of the whole sample and the fraction $>63 \mu\text{m}$.

- SIEVING

Soft sediments

Tip the sample into a $63 \mu\text{m}$ sieve.

Gently wash with tapwater until no more fine-grained sediment is left.

Transfer the residue from the sieve into a saucepan and dry in an oven at not more than 50°C .

Cohesive sediments

Place in a saucepan and dry in an oven for 24 hours.

While hot, cover with a 10% solution of Calgon and allow to stand for 24 hours.

Sieve as above.

Consolidated mudrock samples

Place the sample in a saucepan, dry at 60°C and then break it into pieces of $<1\text{cm}$.

While the sample is hot, cover with paraffin and allow to soak for 24 hours.

Drain off the paraffin into a filter paper to recover for re-use.

Add boiling water and allow to stand.

Sieve as above.

If the sample has not completely broken down, repeat the process.

If the specimens are not clean, soak in a Calgon solution for 24 hours, then wash on a sieve.

- HEAVY LIQUID CONCENTRATION

This must be carried out in a fume chamber. Use rubber gloves and wear a white coat.

If heavy liquid is spilled outside the fume chamber, open the window and leave the room until it has evaporated and dispersed. Avoid inhaling the fumes.

Set up a filter funnel over a beaker on a retort stand.

Place a filter paper labelled with the sample number in the filter funnel.

Put the cool, dry residue from sieving into a separate beaker.

Add about three times its volume of trichloroethylene and stir with a rod.

The microfossils will float to the surface as a scum. Carefully decant both scum and liquid into the filter paper.

Add more heavy liquid to the residue and repeat the process until no more material is floated off.

Remove the filter paper from the funnel and allow it to dry in the fume chamber.

Place the beaker (containing the sediment fraction) on its side and allow to dry overnight.

- STORAGE

Store the flotation in a labelled vial. The sediment residue should be stored in a vial or plastic bag, according to choice/size.

- COUNTING BENTHIC FORAMINIFERAL SAMPLES

Equipment needed:

Gridded picking tray
Fine sable paintbrush (00 or 000)
Small pot of water
Assemblage slide (normally with a black background and divided into 32 cells)
Dilute gum solution.
Stereoscopic binocular microscope with incident light source.

Paint the assemblage slide with the gum solution and allow to dry.

Picking

For samples with many spherical forms, it is desirable to use a sample splitter to divide up the sample. This is more of a problem for planktonic forms.

Quantitative studies

Either the whole sample or a known weight must be picked.

Qualitative studies

To obtain representative proportions at least 250 individuals should be picked.

Procedure

Write the sample number on the assemblage slide.

Tip the sample onto a piece of paper to form a small cone of material.

Use a spatula to take a small amount and carefully sprinkle it over the picking tray.

Place the picking tray under the microscope and, starting in the top left corner, work systematically down the first column picking up the foraminifera with the moistened brush and transfer them to the microscope slide.

Sort into species and place them in separate cells. Because the glue is water soluble, they will adhere.

Continue picking either until the whole sample or 250 individuals have been picked.

If the observer is familiar with all the species, then it may be adequate to just count the numbers of each without mounting them on a slide.

Data

Relative abundance - % of each species.

Absolute abundance - per gm of sediment or per cm³ of sediment.

KEY REFERENCES FOR IDENTIFICATION

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REFERENCES FOR STATISTICAL ANALYSIS

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METHODS OF DATA-INTERPRETATION

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DINOFLAGELLATE CYSTS (Dr I Harding)

1 Sample collection protocols for dinoflagellate cysts.

1.1 Recent sediments

Dinocysts can be extracted from recent unconsolidated sediments collected by standard means (see foram sample collection methods for examples).

1.2 Ancient sediments

Dinocyst can be extracted from many types of ancient sediments, but those most likely to produce good yields are fine-grained, dark coloured sediments such as silts and mudstones, oil shales, etc.. Taking care to avoid contamination, fresh, unweathered samples should be collected in labelled plastic sample bags, and the sample information should be duplicated on a paper label enclosed within the bag. Twice as much sample as is needed for preparation should be collected. For dark mudstones of Mesozoic or Tertiary age this means collecting around 50g of sediment - although in the case of organic-rich oil shales, sometimes as little as 0.1g of sediment needs to be processed to yield a rich assemblage. Coarse-grained sediments (e.g. sandstones) are unpredictable in terms of yield, the larger the grainsize the more likely that the dinocysts will have been winnowed out and deposited elsewhere, thus much larger samples need to be collected and processed. Chemical sediments such as carbonates may yield exceptionally well preserved 3D specimens or nothing at all (as is also the case with evaporites), and again a larger sample size is advisable.

2 Sample processing methods for dinoflagellate cysts.

For recent unconsolidated samples it is possible to disperse the sediment using solutions of Calgon (sodium hexametaphosphate), sieving and then picking out individual specimens with a micro pipette. However, this is laborious, given the average size of a dinocyst is <60µm in diameter, and acid digestion ('palynological') techniques are normally employed:

The dried sediment weighed precisely and crushed into <1cm dia. pieces, placed in a polythene beaker and gradually immersed in 10% hydrochloric acid (taking care with calcareous samples, as these may effervesce spectacularly), and left to react at last overnight. Following the completion of the reaction (additional acid may be added), the supernatant is decanted and the sample rinsed several times to neutrality (being left to stand and settle under gravity after each washing). Carbonates are now removed from the sample.

Silicates are next to be dissolved by gradually adding 60% hydrofluoric acid to the sample. THIS REAGENT IS EXTREMELY DANGEROUS AND IS ONLY USED IN STRICTLY CONTROLLED CONDITIONS, INITIALLY UNDER SUPERVISION UNTIL THE PREPARATOR IS FULLY CONVERSANT WITH COSHH-COMPLIANT SAFETY TRAINING. Again the sample is left to react, for up to 3 days, topping the acid up as the reaction proceeds if necessary. If quantitative results are required, spiking of the residue with a known quantity of an exotic can be undertaken (usually tablets of suspensions of an easily recognisable palynomorph such as modern *Lycopodium* - clubmoss - spores or *Eucalyptus* pollen). Once complete the supernatant is again decanted and the sample repeatedly washed to neutrality.

The sample is then boiled in 10% HCl to remove neo-formed fluorides and again washed to neutrality, and sieved through nylon mesh of an appropriate size (generally 20 μ m). At this stage test slides are made up, and the sample examined to determine whether any additional processing is required to produce a 'clean' residue.

This additional sample preparation normally involves oxidation in concentrated nitric acid (or fuming nitric) to remove extraneous 'amorphous organic matter' (AOM) and pyrite, and sonication of the sample with a tunable ultrasonic probe (again to break up the AOM). These stages are rarely used when preparing recent samples as they can bias the species composition of the residues.

Samples may then be strew-mounted with a pipette onto cover slips and left to dry. Once dry the cover slips are inverted and cemented onto glass slides with Elvacite or a similar medium of appropriate refractive index.

Residues are stored in aqueous solution in labelled plastic vials to which a couple of drops of an antifungal agent has been added.

3 & 4: Data collection and representation techniques for dinoflagellate cyst analysis.

Dinocyst residues are normally analysed by the specific determination of 250-300 specimens per sample (to ensure statistical validity) on a stereo-binocular light microscope (Olympus BH-2) by traversing the glass slide systematically and counting all taxa within each field of view up to the required final total. A mechanical point counter is often used to more reliably randomise the specimens counted, as this machine automatically moves the microscope stage a predefined distance, and the specimen lying under the cross-hairs is then identified. Counting may involve the examination of more than one slide per residue.

The resultant counts are usually expressed both as relative abundance data (percentages) and more frequently nowadays also as absolute abundance data. If the residue was spiked with an exotic, it is possible to calculate absolute abundance data of each counted category per gram of sediment (e.g. 100, 000 dinos/g). This is done, at the same time as counting the 300 dinocysts, by making a separate count of the number of co-occurring exotic grains and by ratio-ing the two counts together, using a standard formula.

The same statistical parameters that have been applied in foram dominance and diversity analysis can also be applied to dinocyst analysis, such as the use of the Fisher Index, Information Index, etc.. Transfer function techniques have also recently been applied to dinocyst assemblages of Pliocene age, but this has not been widely followed up. Such techniques as Canonical Correspondence Analysis have been shown to be very powerful tools in the analysis of data from sequences back into the Pliocene, and may be applicable to other Tertiary samples.

However, most palynological studies do not consider the dinocysts in isolation but along with the data collected from an analysis of the rest of the organic residue isolated along with the dinocysts during palynological preparation. The organic residue usually contains many other particulate organic matter components in addition to the dinocysts (this assemblage of organic debris is termed the 'palynofacies'). Also present may be such palynomorphs such as spores and pollen, other algal remains, foram test linings, etc., in addition to structured phytoclasts (black and brown wood, plant cuticle, etc.) and unstructured AOM.

It is usual for most palynologists (those who work on organic-walled microfossils) to conduct counts not only of one particular taxonomic group, but also to undertake complementary counts of the particulate organic matter in the residue (excluding AOM) - again a point count of 300 particles. This provides relative and absolute abundance information on the composition of the whole organic residue, and provides data which then allows comparisons to be made between both the terrestrial and marine organic contributions to the sediment.

Thus a data set of both the palynofacies and individual palynomorph groups is usually available, and various ratios between different categories (such as total marine vs. total terrestrial, oceanic vs. neritic dinocysts, etc.) can provide valuable information concerning such parameters as influence of oceanic waters, pulses of increased runoff, marine productivity, etc..

The application of various statistical techniques to dinocyst data sets is at an early stage of development, and as yet no standard methods have been adopted, given the infancy of the use of dinocysts as palaeoceanographic or palaeoclimatic tools.

5 References

5a Key references for use in making determinations

There is no single reference which covers this enormous and complex subject area, and identification of taxa can only be done after an extensive introductory course and many hours of microscope work!

However, although the following do not specifically deal with the identification of particular taxa, dinoflagellate cyst morphology is covered in meticulous detail in:

EVITT, W.R. (1985). *Sporopollenin dinoflagellate cysts: their morphology and interpretation*. AASP Foundation, Austin, TX. Book in Short Loan, SOC.

The following is a very good introduction to dinoflagellates:

EDWARDS, L.E. (1993). Chapter 7: Dinoflagellates. **IN:** LIPPS, J. H. (ed.) *Fossil prokaryotes and protists*. Blackwell Scientific Publications. 105-127. Short Loan & GY303 Tutor Boxes, SOC.

Dinoflagellate classification is dealt with in the extremely comprehensive (but turgid!):

FENSOME, R.A., TAYLOR, F.J.R., NORRIS, G., SARJEANT, W.A.S., WHARTON, D.I. & WILLIAMS, G.L. (1993). *A classification of modern and fossil dinoflagellates*. Micropaleontology Special Publication, 7. 351p.

There is a series of indices providing summaries of all the latest taxonomic reassignments - the most current version was published last year, but this is not available in the Library:

LENTIN, J.K. & WILLIAMS, G.L. (1989). *Index to genera and species of fossil dinoflagellates*. American Association of Stratigraphic Palynologists, Contribution Series, 20. 473p.

The commercially available DINOSYS database stores several thousand colour images on a taxonomic/systematic database and can be consulted on the computer in Lab G on Level 6.

5b Key data-analysis references

No one reference covers this subject area

SMITH, J. (1998).

TYSON, R.V. (1994). *Sedimentary Organic Matter*. Chapman & Hall, London.

5c Key Ecological References

DALE, B. (1996). Dinoflagellate cyst ecology: modelling and geological applications. **IN:** JANSONIUS, J. & MCGREGOR, D.C. (eds.). *Palynology: principles and applications. Volume 3*. AASP Foundation, Austin, TX. 1249-1275. Short Loan & GY303 Tutor Boxes, SOC.

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DIATOMS (Dr AES Kemp)

Rationale: Why diatoms are important (and also rather fun!)

Quote: “Whatever the outcome of studies on the limiting factors in the various HNLC (High Nitrate Low Chlorophyll) regions and their subsystems, the status of diatoms as key players will not be challenged. The work-horses running pelagic systems are recruited from this algal group: their new production not only fuels the food chains leading to fish but also provides the raw material driving the biological pump and ultimately the great biogeochemical cycles of the ocean. It is time we gained a better understanding of the properties that make diatoms so special.” Victor Smetacek, News and Views, Nature, **391**, p 225 (1998).

1. Sampling techniques

For classical diatom micropalaeontology, small tooth-pick samples are sufficient. More recently sedimentologically-based research has concentrated on the palaeoecological information preserved in laminated sediments using electron microscope methods and related techniques (see Kemp 1996 and references therein).

2. Sample processing

Simple qualitative diatom identifications may be made by preparing a sediment smear slide using Canada Balsam or UV-setting Norland optical adhesive. In most marine sediments this method will permit identification to genus and commonly also to species level. However, for rigorous identification and quantitative analysis, clays, carbonate and organic matter are removed (see 3 below).

3. Counting techniques

Preparation of the sediment for quantitative diatom slides best follows the method of Lange and Berger (1993) with settling to evenly distribute material over cover slips adapted from Bodén (1991). Briefly, each sample is freeze dried and its dry weight determined. Dry samples are placed in 400 ml beakers and boiled in a solution of sodium pyrophosphate and hydrogen peroxide (30%) (to remove organic matter), followed by treatment with hydrochloric acid (to remove carbonate). The samples are then cleaned with de-ionised water and allowed to settle for 8 hours. The supernatant is decanted off and the dilution procedure repeated (this gets rid of clays which otherwise can be a *#%&# nuisance). This dilution/decanting cycle is typically completed four times, then samples are diluted to a known volume. Cover slips of acid-cleaned material are prepared using a settling technique Bodén (1991), and mounted onto slides using Naphrax mounting medium. Diatoms are counted at 1000 x magnification using the counting convention of Schrader and Gersonde (1978), as modified by Laws (1983). Typically 400 specimens per slide would be counted to get a valid determination.

4. Data-representation

Following counting (as per above) diatom valve concentration per gram dry sediment (DVC or 'valves per gramme') is calculated as:

$$DVC = \frac{WD \times AC \times ND}{WS \times AV \times NV \times VS}$$

where WD = weight of sample solution, AC = Area of settling container, ND = number of diatoms counted, WS = weight of dry initial sample, AV = area of microscope view-field, NV = number of view-fields and VS = volume of sub-sample used settling onto cover slips (Bodén, 1991).

5a. Making determinations

The most important thing to remember with diatoms is that size doesn't matter!

When they reproduce a-sexually, they get progressively smaller, so size is not an important factor in taxonomy. Of course some diatoms are always large (e.g. *Ethmodiscus rex*) and some diatoms are always small (e.g. *Minidiscus*). Key references include the spectacularly illustrated Round et al., 1990 (several copies in library) and Hasle and Syvertsen (1997). A particularly irritating thing is that diatom groupies are keen taxonomists and are always changing names and it is a real pain keeping up!

5b. Data-analysis

The normal suite of statistical analytical techniques used in micropalaeontology are used in diatom analysis including factor analysis (e.g. Sancetta, 1995) and transfer functions (Zielinski et al., 1998). Since we can resolve near-monospecific assemblages with the SEM techniques we use at Southampton (which is much more exciting) the section of the course on diatoms does not deal much with these.

5c. Ecological Information

Diatoms are the world's most important primary producer and occur in environments ranging from mud flats and estuaries through to the deep sea. They are uni-cellular algae that produce a shell or frustule of hydrated opaline silica (SiO₂.H₂O). Diatoms are so significant because they reproduce rapidly and out-compete other phytoplankton. They thus dominate marine production in a wide variety of settings. Diatoms reproduce vegetatively by binary fission but also sexually. Classical diatom micropalaeontologists have traditionally been interested in classification rather than ecology and since biological oceanographers have been primarily interested in the ultraplankton of the microbial loop over the past 30 years there is still quite a gulf in our knowledge of diatom ecology. More recently, in the past ten years or so, there has been a resurgent interest in diatoms as it has been pointed out (not least by Goldman, 1988; 1993) that diatoms are the most important phytoplankton in biogeochemical cycling. Another important and relatively recent advance is the realisation from increasingly sophisticated oceanographic observations (sediment traps etc.) that diatoms tend to aggregate and sink massively as part of their normal life cycle (Alldredge and Jackson, 1995 and references therein).

The standard reference on ecology often quoted is Guillard and Kilham (1978) which draws on Margalef (1967) itself based on studies of the Mediterranean. As one might expect, diatoms that commonly occur in coastal waters (e.g. *Skeletonema*, *Chatoceros*) are relatively well studied compared to oceanic diatoms. In temperate latitudes most production appears to occur in a "spring

bloom". Recent research has shown that some diatom species are also adapted to conditions of enhanced water-column stratification where they can grow at depth in a deep chlorophyll maximum (DCM) (Goldman, 1993) or regulate their buoyancy to travel between a deep nutrient source and higher in the euphotic zone (Villareal et al., 1993).

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