European Mountain lake Ecosystems: Regionalisation, diaGnostics & socio-economic Evaluation

EMERGE

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DIATOM SAMPLING FIELD PROTOCOLS AND SAMPLE PREPARATION

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1. WP4 Rationale;

The overall aim of work package 4 is to model species distribution and food web dynamics in relation to environmental (chemical and physical) gradients. Sampling of both living diatom communities (epilithon) and sediment diatom assemblages (top and bottom) will be carried out at all survey lakes within each lake region.

All samples will be prepared by the laboratory who will carry out the diatom analysis. The sites and diatomists responsible for fieldwork and analyses are tabulated in the EMERGE proposal document.

2. Field methods and analysis

2.1; Field methods for diatom epilithon sampling;

Methods for epilithon sampling will follow those used in the ALPE programme. Diatom epilithon is removed from stones, visibly uncontaminated by sediment, taken from c. 40 - 50 cm water depth along a c. 10 - 20 m stretch of shoreline. Sampling sites close to possible point sources of water quality variation, e.g. inflow streams, should be avoided. Stones in shallower water (< 30 cm) should also be avoided since these are more likely to dry out as a result of fluctuations in lake level.

Algal growth on the stone is detached from the whole of the upper surface using a toothbrush and by repeated washings with distilled water from a wash bottle. The sample is collected in a 1 litre capacity polythene water sample bottle, or similar, via a polythene funnel. At least eight stones should be washed into the bottle in this way to give a single mixed sample. Samples should be preserved by addition of a few drops of Lugols Iodine immediately after collection.

2.2; Sediment Trapping;

Sediment traps provide a useful means of investigating the relationship between living diatom communities and the records of these communities in recent sediment (eg. Cameron 1995). The rationale for their use is set out below.

- Traps provide a link in space between diatom communities and lake sediment diatom assemblages.

- Traps enable short term events to be resolved. Where these events give a weak signal being either of too short a duration or of too low an intensity, for example where too few valves accumulate to register in the stratigraphic record, traps may provide the only clear record.

- Traps provide a continuous relative measure of the composition of the diatom mixture arriving at the sediment during any exposure period. They are a simple means of estimating relative diatom productivity as opposed to the measures of standing crop given by sampling living communities.

Sampling intervals and sediment trap sites are indicated in the proposal.

Wet sediment will be examined for live/dead/broken cells prior to cleaning and counting. Wet mounts can be made as for plankton counting using either a microscope slide & coverslip or inverted microscope and counting chamber. This will provide a measure of sediment resupension prior to taxonomic work on cleaned slides. The purpose of this is to gain an approximate idea of sources of sedimenting material eg. from resuspension vs. recently living cells. A percentage count of the classes live/dead/broken cells or valves will be adequate.

For diatom counting of sedimenting material as well as core assemblages we should routinely count chrysophyte cyst numbers so that both cyst and diatom concentrations can be counted and if required cyst to valve ratios can be calculated.

The microsphere technique (Battarbee & Kneen 1982) should be used as the method for calculating fossil concentrations. The ECRC protocol for preparing diatom samples in this way is available on the web (http://www.geog.ucl.ac.uk/~jhope/lab/ecrc40.htm).

2.3 Methods for sediment diatom sampling;
Sediment coring protocols are outlined elsewhere.
Two sediment diatom assemblages will be analysed from the top and bottom of the short core retrieved from each survey lake.

Preparation will follow the methods used in the ALPE / MOLAR project. Preparation and counting of diatoms from sediment cores will follow standard procedures (Battarbee 1986). Cleaned diatoms are identified and counted under oil immersion at a magnification of c. x 1000 or x 1200 usually under phase contrast, bright field or DIC illumination. In the core samples a minimum of 500 valves will be counted in contiguous samples (Renberg 1990).

3. Lab protocols; Preparation of samples for diatom analysis
It is necessary to remove any organic matter from diatom samples in order to make microscopic identification easier. The cleaning process also allows unwanted mineral material to be removed and the concentration of diatoms to be adjusted (a full discussion of diatom analysis is available in Battarbee 1986).

Safety;
Hydrogen peroxide is a very powerful oxidising agent. Contact with the skin should be avoided and rubber gloves and eye protection should be worn when handling it. If hydrogen peroxide comes into contact with eyes, skin or clothing, wash the spillage under running water. Spills on bench tops, floors etc. should also be diluted with water before mopping up with paper towel. Mopping up concentrated hydrogen peroxide with paper towel can cause fires.
Naphrax diatom mountant contains toluene and should always be handled in a fume cupboard.

3.1 Method for the preparation of samples on a hotplate (for eplithon samples);
This method tends to be quicker than the waterbath method (see below) but needs closer supervision. If beakers are allowed to boil dry, diatoms can be difficult to

dislodge from the glass. There is also a risk of explosion when very concentrated, hot hydrogen peroxide is rapidly oxidising samples with a high organic content. For this reason it is essential that the fume cupboard window is fully lowered if the heating samples are left unattended, and that the window is lowered to the safe working height and eye protection worn whilst working at the fume cupboard.

Equipment;
100 or 250 ml beaker for each sample, Hydrogen peroxide (H_2O_2) 30 % (100 volume), Distilled water, hotplate, 50% Hydrochloric acid (HCl), centrifuge tubes, centrifuge.

Digestion procedure;
i)Place the sample into a glass beaker.

ii)Add about 20 mls H_2O_2.

iii) Heat on a hotplate set at 90oC in a fume cupboard until all organic material has been oxidised (1 3 hours).

iv)Remove the beakers from the heat. Add a few drops of HCl (50%) to remove remaining H_2O_2 plus any carbonates and wash down sides of beaker with distilled water.

v) Allow to cool in the fume cupboard (chlorine is generated from the HCl) and pour into centrifuge tubes, leaving any coarse sand in the beaker. Top up with distilled water.

vi) Centrifuge at 1200 rpm for 4 minutes.

vii) Decant off supernatant and resuspend pellet by tapping the base of the tube. Top up with distilled water and centrifuge as before.

viii) Repeat washing process at least three times. Clay may be removed during the last wash by adding a few drops of very weak ammonia solution (1%) to the sample. The clay is then decanted off with the supernatant.

The sample is now ready to make into slides.

3.2 Method for the preparation of samples using a waterbath;

This method is particularly suitable for large numbers of sediment samples (Renberg, 1990). It can also be safely left without any risk of explosion and there is less risk of samples boiling dry. It may not be suitable for samples which tend to react vigorously with hydrogen peroxide, such as large epilithon and epiphyton samples.
In this procedure centrifuging the washed sample may be replaced by settling out in a cool place overnight. This is convenient when handling a large number of samples in glass test-tubes and reduces breakage of fine, filamentous diatoms. However, if the samples are prepared in plastic centrifuge tubes they may be centrifuged if preferred.

Equipment;
Glass test tubes or plastic centrifuge tubes in a rack, water bath filled with distilled water, Hydrogen peroxide (H_2O_2) 30 % (100 volume), distilled water, hotplate, 50% Hydrochloric acid (HCl).

Digestion procedure;
i) Place approximately 0.01 grams of dried sediment (or 0.1 gram of wet sediment) in each tube, weighing to four decimal places if diatom concentrations are to be calculated. Moistening dried sediment with a few drops of H_2O_2 will help its dispersal when the rest of the peroxide is added.

ii)Add 5 mls 30% H_2O_2 to each tube and place in a rack in the water bath (in a fume cupboard) at room temperature. If the sediment does not react violently with the sediment, the temperature of the water bath can be increased to 80oC (or 90oC if using glass tubes). 'Blank' tubes containing only H_2O_2 can be placed at intervals in the rack and analysed to check there is no cross contamination between the tubes during digestion. Evaporation from the water bath is reduced using floating plastic spheres.

iii) Heat samples for 2 - 4 hours, checking the level of H_2O_2 from time to time, until all organic material has been removed. Do not allow the samples to dry. Also keep the water level in the bath topped up with distilled water.
The heater cuts out and a red alarm light comes on if the water level drops too low.

iv) Remove the tubes from the bath and add just 1-2 drops of 50% HCl to each tube, which will eliminate any remaining H_2O_2 and any carbonates. The fizzing which occurs also helps to unstick any diatoms which may have become attached to the side of the test-tube.

v) Top up test-tubes with distilled water and leave to settle out overnight at 4oC. The resulting supernatant liquid is then decanted off and the diatoms resuspended in more distilled water. Alternatively centrifuge samples in plastic tubes as in 4.2 above.

vi) Repeat this washing process four more times, either allowing the diatoms to settle out overnight at 4oC between each wash or by centrifugation. 1-2 drops of weak ammonia (NH_3) solution added to each sample with the final wash will help keep any clays in suspension and will also prevent the diatoms clumping together when making up slides.

3.3. Diatom slide preparation;
Diatom slides are usually made up by allowing the diatom suspension to settle out on a cover slip overnight, as described below. This produces an even spread of diatoms over the cover slip but it can take up to two days. It is possible to speed up the procedure (resulting in lower quality slides) by gently heating the coverslips after the diatoms have been allowed to settle for 30 minutes. This may result in some clumping of the diatoms but the slides can usually still be counted.

Equipment;
Hotplate, round glass cover slips 19mm diameter, glass slides, 1 ml pipettes for each sample, Naphrax diatom mountant , rigid metal tray, distilled water.

Procedure;
i) Dilute the cleaned diatom suspension to a suitable concentration. It takes practice to get the concentration right. The suspension should look neither totally clear or milky. Fine particles in suspension should be just visible when the suspension is held up to the light.

ii) Place metal settling out trays with cover slips in a position where they will not be disturbed, away from dust sources and air currents.

iii) Using the 1 ml pipette, place up to 0.5 ml of well mixed diatom suspension on each cover slip, cover the tray to keep off dust and leave to dry. This may take up to two days.

iv) Heat a hotplate in a fume cupboard to 130oC.

v) Place 1 drop of Naphrax on a glass slide and invert the cover slip with the dried diatoms over the drop.

vi) Heat the slide on the hotplate for 15 minutes to drive off the toluene in the Naphrax.

vii) Allow the slide to cool and then check that the cover slip does not move when pushed with a fingernail. If it does move then the slide will need to be heated a little longer.

4. Taxonomic harmonisation and data format/ transfer

Data transfer formats for diatom analysis will be circulated to the appropriate analytical laboratories and all regional co-ordinators, ensuring that data transfer is standardised. These templates will also be available via the web page.

Taxonomic harmonisation and data transfer will be achieved through workshops (venue to be announced) within a year of receiving the samples. The exchange of diatom material in the form of published references, descriptions, microscope slides, photographs and material from SEM examination will be undertaken.

5. References

Battarbee, R.W. (1986); *Diatom analysis*. In Berglund, B.E. (ed) Handbook of Holocene palaeoecology & palaeohydrology, John Wiley, Chichester.

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Cameron, N.G. (1995). The representation of diatom communities by fossil assemblages in a small acid lake. Journal of Palaeolimnology 14: 185-233.

Renberg, I. (1990); A 12600 year perspective of the acidification of Lilla Öresjön, southwest Sweden. Phil. Trans.R. Soc. Lond. B 327, 357-361