Sampling protocol for AMS radiocarbon dating of terrestrial plant macrofossils

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In contrast to conventional radiocarbon decay-counting, Accelerator Mass Spectrometry (AMS) radiocarbon dating requires sample sizes that are substantially smaller. Generally, a sample size containing about 1 mg organic carbon is needed for AMS radiocarbon dating. It is wise to check with your laboratory before proceeding. Some examples are given below. Smaller samples (0.2 mg C) can be dated but the error can be larger. Besides allowing a better stratigraphic resolution (i.e. slices of 0.5-1 cm instead of e.g. bulk sediment samples using 5-10 cm of the core) we must realise that, when dealing with such small samples, a small amount of contamination will lead to a substantial deviation from the 'true' radiocarbon age. An advantage of AMS dating is that terrestrial plant material (e.g. macrofossils) can be extracted from the sediment and used for dating. In addition, minerogenic sediment often contains sufficient macrofossils for AMS-dating. The use of terrestrial material avoids problems associated with hard-water (or freshwater) reservoir effects in lake sediment and in aquatic plant material. However, the operator needs sufficient botanical skill to be able to identify the plant macrofossils. In general, macrofossils of aquatic plants or mosses should not be dated as they contain a hard-water or freshwater reservoir error (see for example, Olsson 1974, 1983; Törnqvist 1992; Philipsen 2013). In addition, AMS dating is usually faster than bulk-sample dating.

A standard procedure of sample preparation follows the protocol below:

Separation of terrestrial plant macrofossils from the sediment

1. Cut the wet core into suitable slices. Avoid sampling slumps or turbidites as they may include older reworked material.

2. Do not store these samples for more than a few days in the refrigerator (see Wohlfarth et al., 1998). If you cannot proceed immediately, it is best to deep-freeze the samples.

3. Disintegrate the sediment by soaking it in water in a clean, labelled beaker with a lid. If necessary, sediment breakdown can be assisted by adding a small spoonful of tetra-sodium diphosphate-10-hydrate crystals $Na_4P_2O_7 10H_2O$, also known as sodium pyrophosphate, or Calgon water softener. Shake to dissolve the powder and stand overnight or for as long as necessary in the refrigerator. If the sediment is calcareous, treatment with 10% HCl may be desirable; beware of excessive effervescence. If it is humified peat or organic lake sediment, short treatment (e.g.1 hour to overnight) with cold 10% KOH may be desirable.

4. Sieve the sample through a 250µm mesh. (Note! if macrofossil analysis is to be done on the sample(s), sieve through a 125µm mesh to retain small seeds and also zoological remains (e.g. Chironomids, Cladocera etc.) should these be of interest. It may be useful to use a selection of mesh sizes. If macrofossil material is sparse, save the sediments >125µm in addition to the larger fractions.

5. Store the sieved material under water in clean beakers with lids, both clearly labelled, or in sealable plastic bags, in the refrigerator. If you cannot proceed immediately, deep-freeze the samples.

Selection of material for dating

1. Treat only one sample at a time to avoid confusion. Label all containers carefully.

2. Disperse a little of the material in water in a clean shallow (petri) dish. Under a stereo-microscope, magnification ca. 12x, pick out the terrestrial plant fragments with soft (entomological) forceps. It may be useful to separate the different types, e.g. mosses, leaves, seeds, etc. for easier identification, or selection of one sort of material. The material for dating should be identified and only that of terrestrial origin should be selected.

3. Remove as much sediment or other material as possible adhering to the selected macrofossil material with a small brush or forceps.

4. Under the microscope, pick out the remains once more, carefully avoiding sediment and any dust, hairs etc. and place them in clean tap water in a new clean container. This is a washing process.

5. Pick out the remains again and place them in a dry, dust-free box or glass. Let the sample dry, covered, at room temperature or deep-freeze the remains in clean water and freeze dry them.

6. Weigh the dried sample to ensure you have enough material for dating, as required by your dating laboratory. It is usually best to submit at least 3 mg to produce sufficient carbon, although some laboratories can use as little as 0.5 mg dry material. On such small samples, the measurement of $\delta^{13}C$ may be impossible. In general, the carbon content is half the dry weight of the sample and 1 mg carbon is needed for reliable AMS dating.

7. Control the dry sample once more for dust or clothing hairs. Wool hairs for example will make the sample 'younger'; synthetic oil-derived hairs will make the sample 'older'. With such small samples, it is very important to do this.

8. Sterilize a glass vial at 600^{o}C for 3 minutes. Cool it. Place the clean sample in it. Label both the vial and the lid. Glass containers are preferable, as plastic suffers from static electricity which makes the fragments jump about.

9. The dry samples can be stored as necessary in a dark cold-room until they are sent to the dating laboratory.

Steps 2-4 can be carried out in a laminar-airflow chamber to reduce the chances of contamination by air-borne dust and clothing hairs.

Important: As AMS samples are very small, small amounts of fungal or bacterial biomass may easily contaminate the samples and result in an erroneous age. Therefore, care should be taken to avoid this at all stages in the preparation; by proceeding rapidly, and by storing the samples in a dark refrigerator between stages. If delay is unavoidable, the material should be deep frozen. Samples should never be left in water at room temperature, as fungal growth can be extremely rapid. Samples that have been stored in this way or in organic liquids (e.g. alcohol, glycerol etc.) should **not** be used for dating or $\delta^{13}C$ measurements. Working conditions should be as clean as possible, and care should be taken to remove all dust, hairs, etc. from containers before use, and to control the samples for these contaminants as outlined above. With larger fragments, such as wood, do not handle them with your fingers.

Before sending off the samples for dating, note the following data in your lab journal and provide the information to the dating laboratory:
- site name
- core ID, segment, date of coring
- core depth and span of sample
- date of macrofossil processing, treatment
- the plant material that is used for dating (e.g. 5 *Pinus* needles, 20 *Betula* fruits, etc.)
- dry weight of submitted material
- expected age

When reporting radiocarbon ages in publications, the following information should be provided:
- sample depth or code
- dating lab sample number
- material dated and weight
- weight of carbon
- radiocarbon age ± 1sd of measurement
- calibrated age (as cal yr BP, with reference to program and calibration data-set used)
- $\delta^{13}C$ if measured

Calibration of Radiocarbon Dates

Radiocarbon ages need to be calibrated to calendar years, using the calibration dataset, currently IntCal13. This is important for archaeological samples, for the correlation of events, and if an accumulation rate of a sediment sequence is needed. Radiocarbon dates should be calibrated using either IntCal13 for terrestrial northern hemisphere material (Reimer et al., 2013), Marine13 for marine material (Reimer et al., 2013), or SHCal13 for southern hemisphere dates (Hogg et al., 2013), Calibrated dates should be indicated by cal yr BP (BP is Before Present, which for radiocarbon dates refers to 1950 CE).

Calibration of radiocarbon dates can be done with various programs. The most commonly used is CALIB 7.1 (Stuiver et al. 2020; http://calib.org). Many programs for age-depth modelling incorporate date calibration.

Several programs are available for making an age-depth timescale through a sequence. The most commonly used are Bacon (Blaauw and Christen, 2011), BChron (A, Parnell, https://rdrr.io/cran/Bchron/), and OxCal (C. Bronk Ramsey, https://c14.arch.ox.ac.uk/oxcal.html).

Computing is normally done in R (https://www.r-project.org/). For more information on calibration and age-depth modelling, see Blaauw and Heegaard (2012).

References

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Blaauw, M. and Heegaard, E. 2012. Estimation of age-depth relationships. Chapter 12 in Tracking Environmental Change using Lake Sediments; Volume 5, Data Handling and Numerical Techniques. Ed. by H.J.B. Birks, A.F. Lotter, S. Juggins, and J.P. Smol. Springer, pp 379-413.

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Some dating laboratories

Beta Analytic, Miami: https://www.radiocarbon.com/required-carbon-dating-sample-sizes.htm

Belfast University, N. Ireland: http://www.chrono.qub.ac.uk/

Poznan Radiocarbon Laboratory, Poland: https://radiocarbon.pl/en/types-of-samples-suitable-for-dating/

Waikato, New Zealand: https://radiocarbondating.com/sample-submission/sample-type-and-size-requirements

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