

## 14. CARBON AND OXYGEN ISOTOPE ANALYSIS OF LAKE SEDIMENT CELLULOSE: METHODS AND APPLICATIONS

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### Introduction

Carbon and oxygen isotope analysis of lake sediment cellulose is a recently developed paleolimnological approach that is gaining increasing usage, especially in carbonate-free sedimentary systems. As with carbonate-based paleolimnological investigations (see Ito, this volume), lake sediment cellulose can provide a record of lake paleohydrology. As a result, studies incorporating this technique typically aim to address the following research questions:

1. How has the water balance of the study lake varied during the past?
2. What does the inferred paleohydrologic record suggest about past changes in climatic conditions?
3. What is the relationship between past hydrologic change and lake or watershed carbon cycling?

Lake sediment cellulose has been used as an archive of paleoenvironmental information in diverse geographic and ecoclimatic settings. These include Great Lakes of both North



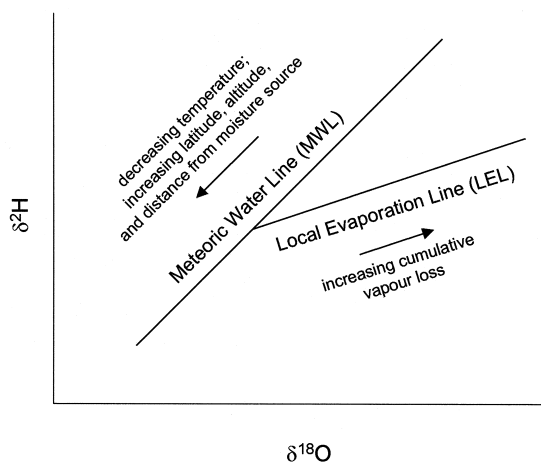


Figure 1. The oxygen and hydrogen isotope compositions of precipitation and surface waters commonly define two linear relations in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  space. Precipitation plots along a meteoric water line (MWL), while surface waters having undergone vapour loss plot on a local evaporation line (LEL).

America (Ontario; Duthie et al., 1996; Wolfe & Edwards, 1998; Wolfe et al., 2000c) and East Africa (Victoria; Beuning et al., 1997), smaller lakes in temperate regions of North America (Edwards & McAndrews, 1989; Padden, 1996; Padden et al., 1996; Wolfe et al., 2000a), in the circumpolar arctic and subarctic (MacDonald et al., 1993; Edwards et al., 1996; Wolfe et al., 1996, 1999, 2000b) and in the subtropical Andes (Abbott et al., 2000), as well as ancient Glacial Lake Agassiz (Buhay & Betcher, 1998). While substantial progress has been made over the last decade in methodological development and interpretation of stable isotope data from lake sediments, various intriguing questions remain to be addressed in future investigations.

This chapter details the technical procedures used to prepare and analyze lake sediment samples for cellulose carbon and oxygen isotope composition, considers important factors in the interpretation of cellulose isotope data, briefly highlights key results from recent applications, and outlines future research needs and directions.

### Stable isotope tracers in lake waters — $^{18}\text{O}$ , $^2\text{H}$ , $^{13}\text{C}$

Phase changes during the passage of water through the hydrologic cycle cause variations in the respective abundances of  $^{18}\text{O}$  and  $^2\text{H}$  relative to  $^{16}\text{O}$  and  $^1\text{H}$ , because of slight differences in the volatilities of the different water molecules. This isotopic fractionation results in characteristic isotopic labeling of meteoric waters. A key feature of this natural labeling is the existence of strong linear relations (“Meteoric Water Lines”, MWLs) commonly observed between  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values in precipitation falling at different times of the year. MWL relationships arise because of isotopic rain-out of atmospheric vapour masses and temperature-dependent fractionation effects in clouds (Fig. 1). As a result, decreasing temperature at the site of condensation and increasing latitude, altitude, and distance from

moisture source (continentality) will generally result in progressively decreasing  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values in precipitation (Dansgaard, 1964; Rozanski et al., 1993).

Preferential loss of molecules containing the common light isotopes,  $^{16}\text{O}$  and  $^1\text{H}$ , during subsequent evaporation from surface waters enriches the remaining water in the heavy isotopes,  $^{18}\text{O}$  and  $^2\text{H}$ , causing displacement from the MWL in  $\delta^{18}\text{O}$ - $\delta^2\text{H}$  space (Fig. 1). Surface waters within an individual catchment frequently plot along well-defined Local Evaporation Lines (LELs) having slopes generally between 4 and 7, largely depending on the relative humidity, and offset from the MWL in relation to the amount of cumulative vapour loss (Craig & Gordon, 1965; Gonfiantini, 1986).

In contrast to lake water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , which are largely controlled by physical mechanisms, the  $\delta^{13}\text{C}$  of dissolved inorganic carbon (DIC) in lake water may be strongly mediated by in-lake biological processes, as well as exchange with atmospheric  $\text{CO}_2$  (McKenzie, 1985; Quay et al., 1986; Herczeg, 1987; Herczeg & Fairbanks, 1987; Lee et al., 1987). During photosynthesis, phytoplankton preferentially consume  $^{12}\text{CO}_{2(\text{aq})}$ , leaving the remaining  $\text{CO}_{2(\text{aq})}$  relatively enriched in the heavy carbon isotope,  $^{13}\text{C}$ . Recent work suggests that many lakes may act as sources rather than sinks of atmospheric  $\text{CO}_2$  (Cole et al., 1994). However, in productive lakes where diffuse influx of atmospheric  $\text{CO}_2$  does occur, this input replenishes  $\text{CO}_{2(\text{aq})}$  withdrawn by phytoplankton and may serve to amplify the biologically-induced  $^{13}\text{C}$ -enrichment of the epilimnetic DIC pool.

Decaying plant material that sinks below the photic zone releases  $^{13}\text{C}$ -depleted DIC, which may be reincorporated into phytoplankton tissue upon circulation and mixing of lake water. In lakes where the carbon isotope effects of photosynthesis and respiration are not as pronounced, river and groundwater input of DIC may exert a greater influence on lake water DIC  $\delta^{13}\text{C}$  (Rau, 1978). Catchment-derived DIC  $\delta^{13}\text{C}$  may potentially span a wide range of values ( $\approx -30$  to  $0\text{‰}$ ) reflecting influx of dissolved  $\text{CO}_2$  from the decomposition of  $^{13}\text{C}$ -depleted soil organic matter (in areas devoid of carbonate) and the chemical weathering of  $^{13}\text{C}$ -enriched carbonate terrane (Boutton, 1991b).

Close linkage may occur between seasonal cycles of lake water  $\delta^{18}\text{O}$  and DIC  $\delta^{13}\text{C}$ . For example, lakes may develop progressively increasing values of epilimnion  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  during the open-water season due to the combined effects of evaporative  $^{18}\text{O}$ -enrichment and  $^{18}\text{O}$ -enriched summer precipitation in addition to productivity-driven  $^{13}\text{C}$ -enrichment and atmospheric  $\text{CO}_2$  drawdown, respectively. Variations through time in the amplitude and pattern of these seasonal cycles may be manifested as long-term trends, recorded in the lake sediments. These variations result from changes in numerous climatic and environmental factors including the carbon and oxygen isotope composition of source waters, relative humidity, residence time, lake nutrient status, and physical characteristics of the catchment such as vegetation, hydrology, geology, and soil development.

Thus, isotope-based paleolimnological and paleoclimatological reconstructions have relied mainly on carbon and oxygen-isotope signals preserved in carbon- and oxygen-bearing inorganic and organic matter produced within the lake water and preserved in bottom sediments. Carbonates of both authigenic and biogenic origin have been used extensively in hard-water lakes, especially at low and mid-latitudes (Ito, this volume), whereas organic matter and cellulose have proven to be useful archives in carbonate-deficient soft-water lakes. Increasing attention is now being focused on nitrogen isotope composition of bulk organic matter (Talbot, this volume) and the hydrogen isotope composition of kerogen (Krishnamurthy et al., 1995; Meyers & Lallier-Vergès, 1999) and aquatic plant lipids

(Sternberg, 1988; Buhay, 1997) as additional isotopic paleo-nutrient and paleohydrologic tracers, respectively.

### Historical development

Cellulose is the most abundant bio-molecule in nature, occurring in the cell walls of higher and lower plants primarily in stalks, stems, trunks and all woody components of plant tissues (Ott et al., 1954). Cellulose is also a structural component of the cell walls of some algae, comprising from 1–10% of the dry weight of the organism (Prescott, 1968; De Leeuw & Largeau, 1993). Cellulose in algal cell walls can exist as native cellulose (cellulose I) or as amorphous forms or single chain molecules (Ott et al., 1954; Kreger, 1962). Primary algal groups in which cellulose has been identified in freshwater species are Charophyta, Chlorophyceae, Desmidiaceae and Zygnematales, including such common genera as *Cladophora*, *Closterium*, *Nitella*, *Oocystis*, *Pediastrum*, *Spirogyra*, and *Botryococcus* (Kreger, 1962; Prescott, 1968; Tsekos, 1999). Although some studies suggest otherwise (Chapman, 1962), there is no conclusive evidence for cellulose in blue-green algal cell walls (Kreger, 1962).

Within lake sediments, cellulose may be preserved as identifiable algal cells (e.g., *Botryococcus* in Lake Victoria (Beuning, unpublished data)), within zooplankton fecal pellets (Edwards, 1993), or as amorphous organic matter. The chemical structure of cellulose contains carbon, hydrogen, and oxygen, each a potential archive of isotopic information. While the ratio of carbon, oxygen and carbon-bound hydrogen isotopes is “locked in” with death of the cellulose-synthesizing organism, oxygen-bound hydrogens are exchangeable (Sternberg, 1989a). As a result, hydrogen isotope analysis of cellulose requires nitration or equilibration preparative procedures to control exchangeable hydrogen. Moreover, strong species-dependent effects on cellulose-water hydrogen isotope fractionation have precluded its use as a paleo-isotopic archive (e.g., Sternberg, 1988).

Carbon and oxygen isotope analysis of cellulose in lake sediments was first conducted by Edwards & McAndrews (1989) to investigate the Holocene paleohydrology of a small lake in southern Ontario, Canada. Two important conclusions were drawn from this study. Firstly, the fine-grained cellulose fraction in offshore sediments appeared to be dominantly aquatic in origin. This interpretation was based primarily on coherent isotopic relations between the surface sediment cellulose and the modern lake water as well as overlapping  $\delta^{18}\text{O}$  records from two widely separated sediment cores. Secondly, lake sediment cellulose  $\delta^{18}\text{O}$  could be used to trace lake water  $\delta^{18}\text{O}$  history by applying a similar cellulose-water fractionation factor as had been shown to exist between terrestrial cellulose and leaf water (DeNiro & Epstein, 1981; Sternberg et al., 1984; Edwards et al., 1985). This study, combined with prior isotopic investigations in southern Ontario (Edwards et al., 1985; Edwards & Fritz, 1986, 1988), led to the conceptual development of analogous oxygen isotope relations between terrestrial cellulose, lacustrine inorganic authigenic carbonate and lacustrine aquatic cellulose, and the oxygen isotope composition of the respective source waters (Fig. 2).

For terrestrial cellulose:

$$\delta^{18}\text{O}_{\text{cell}} = \delta^{18}\text{O}_{\text{mw}} + \varepsilon_{\text{evap}} + \varepsilon_{\text{cell-leafwater}}, \quad (1)$$

where  $\delta^{18}\text{O}_{\text{cell}}$  is the isotopic composition of terrestrial cellulose,  $\delta^{18}\text{O}_{\text{mw}}$  is the isotopic composition of local meteoric water,  $\varepsilon_{\text{evap}}$  is the isotopic enrichment due to combined kinetic

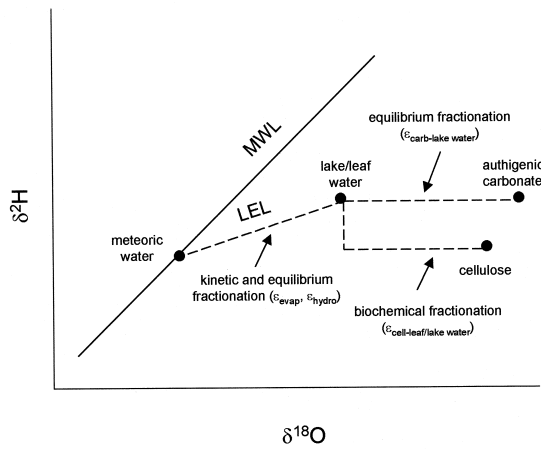


Figure 2. Location of authigenic carbonate and cellulose in  $\delta^2\text{H}$ - $\delta^{18}\text{O}$  space and associated fractionation effects.

and equilibrium effects during evapotranspiration, and  $\epsilon_{\text{cell-leafwater}}$  is the net biochemical enrichment between cellulose and leaf water.

For lacustrine inorganic authigenic carbonate:

$$\delta^{18}\text{O}_{\text{carb}} = \delta^{18}\text{O}_{\text{mw}} + \epsilon_{\text{hydro}} + \epsilon_{\text{carb-lakewater}}, \quad (2)$$

where  $\delta^{18}\text{O}_{\text{carb}}$  is the isotopic composition of lacustrine inorganic authigenic carbonate,  $\delta^{18}\text{O}_{\text{mw}}$  as in (1),  $\epsilon_{\text{hydro}}$  is the isotopic enrichment between lake water and meteoric water due to hydrologic factors which are commonly dominated by evaporative enrichment effects, and  $\epsilon_{\text{carb-lakewater}}$  is the temperature-dependent enrichment that occurs between carbonate and lake water.

For lacustrine aquatic cellulose:

$$\delta^{18}\text{O}_{\text{cell}} = \delta^{18}\text{O}_{\text{mw}} + \epsilon_{\text{hydro}} + \epsilon_{\text{cell-lakewater}}, \quad (3)$$

where  $\delta^{18}\text{O}_{\text{cell}}$  is the isotopic composition of aquatic cellulose,  $\delta^{18}\text{O}_{\text{mw}}$  as in (1),  $\epsilon_{\text{hydro}}$  as in (2), and  $\epsilon_{\text{cell-lakewater}}$  is the isotopic enrichment that occurs during cellulose synthesis and is identical to  $\epsilon_{\text{cell-leafwater}}$ .

Thus it was proposed that, with stratigraphic analysis of lacustrine aquatic cellulose, reconstruction of lake water  $\delta^{18}\text{O}$  could be used to document shifts along a LEL and/or MWL from which paleohydrologic and paleoclimatic information may be derived.

## Methods

### Sample preparation

Methods for lake sediment sample preparation for cellulose carbon and oxygen isotope analyses have been developed at the Environmental Isotope Laboratory (EIL), University

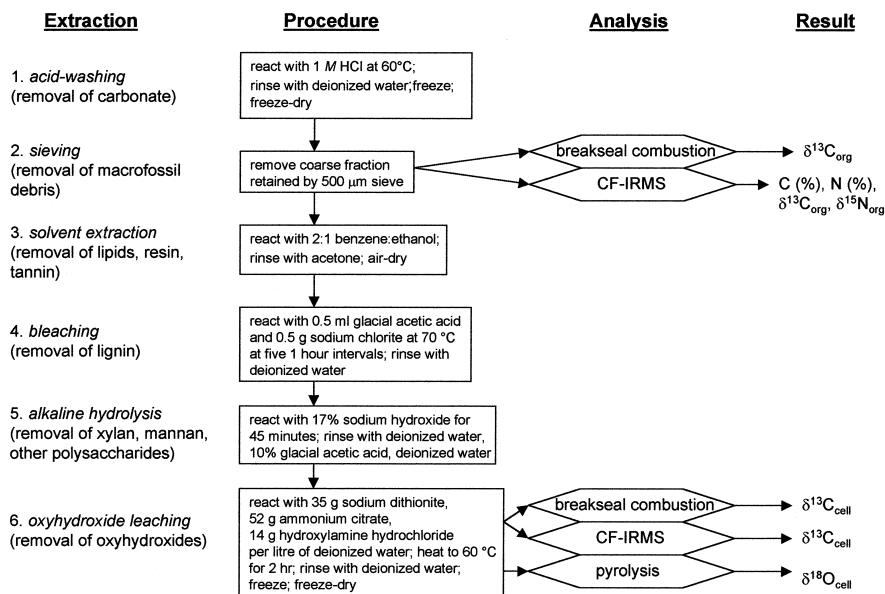


Figure 3. Flow diagram illustrating steps involved in preparing lake sediment for cellulose carbon and oxygen isotope analysis.

of Waterloo (Heemskerk & Diebolt, 1994; Edwards et al., 1997; Elgood et al., 1997). These techniques closely follow those of Green (1963) and Sternberg (1989a) for extracting cellulose from wood powder, as indicated in the inaugural study of Edwards & McAndrews (1989). Modifications have been introduced to accommodate the much finer grain size typical of sediments and for removal of other potentially interfering geochemical fractions (Fig. 3). Preliminary sample treatment involves acid-washing to dissolve carbonate material and dry sediment sieving to remove macrofossil debris after which analysis of bulk carbon and nitrogen elemental and isotope composition may be conducted. Sediment cellulose preparation on the acid-washed residue is generally a three-part process involving sequential extraction of non-cellulose organic components. An additional leaching step removes oxyhydroxides. All extractions are performed in a fumehood and normal laboratory safety precautions should be adhered to (e.g. lab coat, gloves, dust mask and safety glasses should be worn). Batches of 10 or more may be processed simultaneously, depending on the size of the bottles used and space restrictions in the fume hood and water bath. Note that the use of a centrifuge significantly reduces processing time and may contribute to less-corroded cellulose residue by minimizing sample exposure to the solutions in the bleaching and hydrolysis steps. Details of methods used at the EIL are provided below.

#### A) Acid-washing (removal of carbonate)

1. Place sample in a labeled 75 ml test tube and add 1 M (approximately 8% by volume) HCl. Stir and cover with watch glass. Place in water bath for a minimum of two hours at 60 °C.

2. Check the pH to ensure samples are acidic. If not acidic or visible reaction occurs upon agitation, pour/aspirate supernatant and add fresh HCl and repeat step #1. If acidic, pour/aspirate supernatant, fill with deionized water and stir.
3. Leave standing until sediment settles or centrifuge and pour/aspirate supernatant. Rinse repeatedly with deionized water until neutral.
4. Pour/aspirate supernatant, freeze, freeze-dry, and store in labeled, clean, dry vial.
5. Following removal of carbonate and freeze-drying, lake sediment samples are sieved to  $< 500 \mu\text{m}$  (if necessary) to eliminate microfossil plant debris (which may be of terrestrial origin). Scattered fragments may be removed with tweezers.

B) Solvent extraction (removal of lipids, resins, tannin)

1. Extract 2–5 g of freeze-dried sediment (depending on organic content of the sediment) in a covered 125 ml glass (or Teflon) wide-mouth, screw-top bottle with about 100 ml of 2 : 1 benzene : ethanol, swirling the solution occasionally. Decant/aspirate the solution after 48 hours. If the liquid is deeply coloured (i.e. darker than weak tea), repeat the extraction with fresh solvent for an additional 24 to 48 hours.
2. Add about 100 ml of acetone and replace cover. After 24 hours, decant/aspirate and allow samples to air-dry in the fumehood.

C) Bleaching (removal of lignin)

1. Add about 100 ml of deionized water to the air-dried sample and place in a water bath at  $70^\circ\text{C}$ . Add 0.5 ml of glacial acetic acid, followed by 0.5 g of sodium chlorite, stir, and cover.
2. After one hour, add fresh aliquots of acetic acid and sodium chlorite (always adding the acetic acid first), and stir. Repeat 5 times or until sediment residue is a pale grey to yellowish-grey colour.
3. Allow sediment residue to settle, decant/aspirate supernatant liquid, and re-fill with deionized water. Repeat 5–10 times, or until odour of the bleach solution fades, to completely displace bleach solution. Do not test the odour until the solution is thoroughly diluted.
4. After the final dilution, decant/aspirate to within 1 cm of the residue.

D) Alkaline hydrolysis (removes xylan, mannan, other polysaccharides)

1. Add about 100 ml of 17% sodium hydroxide solution to the wet sample. Let stand for 45 minutes, decant/aspirate, and fill with deionized water. Rinse with deionized water 3–4 times or until solution is near neutral.
2. Dilute once with 10% acetic acid and allow to stand for 15 minutes. Thoroughly wash the residue by repeatedly rinsing with deionized water until the odour of acetic acid fades. (Test the pH with indicator paper to confirm that it matches that of the deionized water).
3. After the final dilution, decant the water to within 1 cm of the grey cellulose residue.

## E) Oxyhydroxide leaching (removes oxyhydroxides)

1. Oxygen isotope results may be affected (and the nickel pyrolysis tube contaminated) if iron or manganese oxyhydroxides are present (often indicated by distinctive reddish or orange-brown colour of the final cellulose concentrate) and should be removed using the following leach solution:

Dissolve in 1 L of deionized water:

35 g sodium dithionite (also called sodium hydrosulfite)

52 g ammonium citrate

14 g hydroxylamine hydrochloride

2. Extract oxyhydroxide-contaminated cellulose concentrate with about 75 ml of leach solution in water bath at 60 °C for 2 hours. Remove and let stand at room temperature for 24 hours.
3. For samples with solution that visibly discolour, decant/aspirate and replace with fresh dose of leach solution and let stand at room temperature for 24 hours. If no discolouration is visible, decant/aspirate and replace with deionized water. Let stand for 24 hours.
4. If colour remains (or returns to those samples which have been rinsed with deionized water), repeat with fresh dose of leach solution and let stand for 24 hours. For samples in deionized water that continue to show no colour, wash by repeated dilution and decanting/aspirating with deionized water.
5. After the final dilution, decant the water to within about 1 cm of the grey cellulose residue, freeze, and freeze-dry.
6. After the sample is thoroughly dry, transfer to a clean, dry, labeled vial for subsequent carbon and oxygen isotope analysis.

*Analysis*

Carbon isotope analysis on lake sediment cellulose may be performed by routine breakseal combustion (Boutton et al., 1983; Boutton, 1991a) or by continuous flow — isotope ratio mass spectrometry (CF-IRMS). Comparison of lake sediment samples analyzed by both methods at EIL show excellent agreement.

Oxygen isotope analysis of lake sediment cellulose has been performed mainly by Ni-tube pyrolysis in re-sealable nickel vessels, as first described in Edwards et al. (1994), although a HgCl<sub>2</sub> technique has also been used (Sternberg, 1989b; Sauer & Sternberg, 1994; Sauer et al., 2000). Vessels currently in use at the EIL have undergone minor design modifications since those described by Edwards et al., including the use of stainless-steel rather than nickel Cajon VCR<sup>®</sup> metal gasket face seal fittings (Fig. 4). With the original vessel design, which included a tapered nickel plug fitted to a beveled nickel seat (see Fig. 1 in Edwards et al., 1994), cleaning of the interior of the vessels rapidly wore down the sealing surface, eventually resulting in gas leakage. More recently, changes in vessel design has enabled better sealing and significantly improved the durability of the vessels. While this new design does result in exposure of a small amount of stainless steel to the

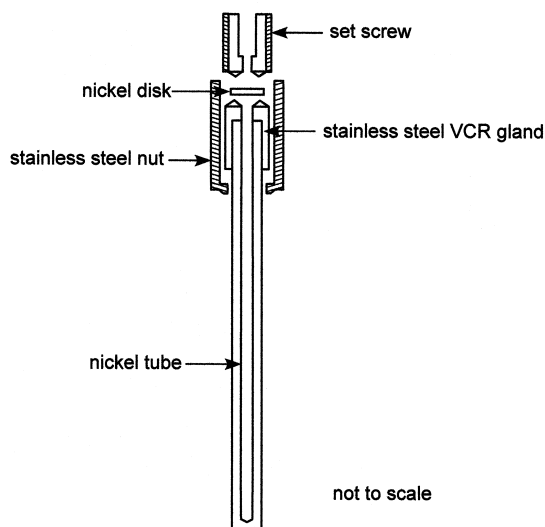
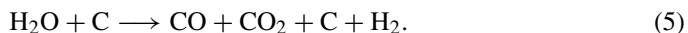
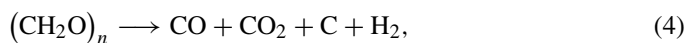


Figure 4. Current vessel design used for pyrolysis at the University of Waterloo.

interior of the vessels, there is no evidence to suggest that this has any apparent effect on  $\delta^{18}\text{O}$  results.

Vessels are loaded with terrestrial cellulose, lake sediment cellulose, or water and graphite (as a carbon source) and sealed in an argon atmosphere, followed by heating (pyrolysis) at  $1050\text{ }^\circ\text{C}$  in an evacuated quartz tube. The addition of an encapsulating quartz tube has been incorporated to prevent repeated oxidation of the outer nickel surface and to simplify the cleaning procedure. Heating has been increased from  $950\text{ }^\circ\text{C}$  used in the original experiments to  $1050\text{ }^\circ\text{C}$  to account for the insulation of the quartz tube (see Motz et al., 1997).

The pyrolysis reactions can be described by the general (unbalanced) equations:



Hydrogen in the samples forms  $\text{H}_2$  gas that diffuses out through the nickel vessel walls and which may be collected and used to determine  $\delta^2\text{H}$  (Motz et al., 1997). Oxygen is incorporated into  $\text{CO}$  and  $\text{CO}_2$  and contained within the vessels. Oxygen isotope analysis is performed on the  $\text{CO}_2$  after cryogenic purification on a vacuum extraction line. Quantitative recovery of oxygen from  $\text{CO}$  is considered unnecessary as the oxygen isotope composition in  $\text{CO}$  and  $\text{CO}_2$  appears to equilibrate at high temperatures with negligible fractionation, which is then retained during cooling to room temperature (Edwards et al., 1994).

The technical procedure for Ni-tube pyrolysis described below is based on equipment designed at the EIL. Modifications may be introduced to adapt to existing laboratory equipment, vacuum extraction lines, etc.

## A) Vessel cleaning

Prior to use, pyrolysis vessels must be carefully cleaned to remove all debris remaining from the previous sample.

1. Clean the interior of vessel using a stainless steel brush (1/4" diameter). Gently tap out debris. Care must be taken not to score the raised circular seats at the opening of the vessel as this will result in a poor seal and loss of gas during pyrolysis.
2. Flush interior of vessel with acetone and allow to air-dry.
3. Place clean vessels in oven at 60 °C or keep in a desiccator under vacuum. During periods of prolonged non-use, the pyrolysis vessels should be kept in a desiccator to minimize oxidation of nickel surfaces.

## B) Loading vessels (with water or cellulose samples) and pyrolysis

Although samples are vacuum-dried during the process of cellulose extraction (see above), it has been demonstrated that moisture is readily absorbed from the air (Edwards et al., 1994). Furthermore, due to the small sample size, any moisture can have a significant effect on the final analysis. It is therefore important to adhere to the following procedure designed to remove all traces of atmospheric moisture that may cause sample contamination.

1. Place samples in desiccator, attach to freeze dryer or extraction line and evacuate. Leave samples under vacuum for a period of at least 12 hours.
2. Clean and dry vessels as described above.
3. For water samples, load pre-baked, dry carbon (1 mg/1  $\mu$ lH<sub>2</sub>O) into vessels, cover with tinfoil and place in desiccator. Five ml of H<sub>2</sub>O typically produces 1.5 to 2 cc of CO<sub>2</sub>. For cellulose samples, load into vessels, place in desiccator chamber and cover with tinfoil. Gloves should be worn to prevent contamination of sealing surfaces. For lake sediment samples with very low organic content, it is important not to overload vessels as the pyrolysis reaction will not go to completion. Typically, 3–5 mg are used for pure terrestrial cellulose samples (producing 1.5 to 2 cc of CO<sub>2</sub>) and roughly 20 mg are used for lake sediment cellulose (producing roughly 0.5 cc of CO<sub>2</sub>). The amount of lake sediment cellulose concentrate required to produce sufficient CO<sub>2</sub> will vary according to the amount of refractory mineral matter.
4. Place vessels in desiccator and attach to freeze dryer or extraction line and evacuate. Leave vessels under vacuum for a period of at least 12 hours.
5. Close desiccator and remove from freeze dryer or extraction line. Place desiccator in glovebox.
6. Flood glovebox with argon. Allow sufficient time for glovebox to be totally flushed with argon.
7. Open desiccator to argon atmosphere.
8. Place nut in holder and transfer vessel from desiccator to holding rack in glovebox. Gloves should be worn to prevent contamination of sealing surfaces.

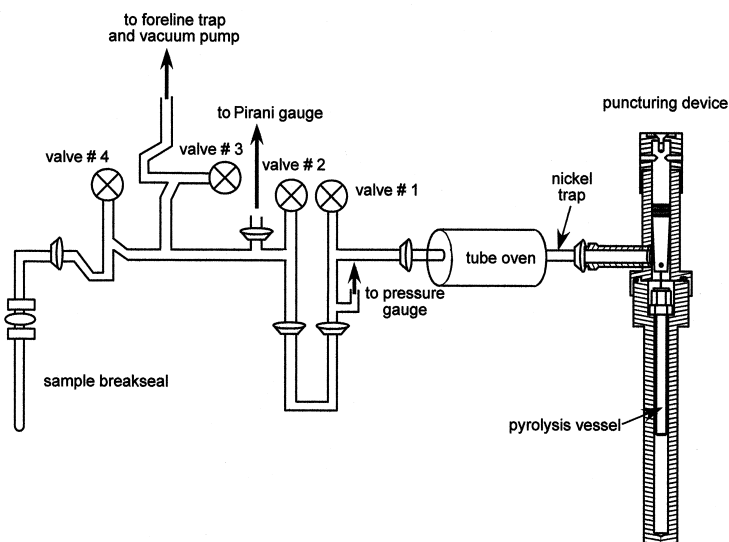


Figure 5. Schematic of CO<sub>2</sub> extraction line used at the University of Waterloo.

9. Flush vessel with thin stream of argon to further ensure that all atmosphere is displaced. Note that pure cellulose is easily blown out of vessel! (For cellulose samples, go to step 11).
10. Lower syringe as far as possible into the vessel and inject water sample.
11. Quickly place nickel disk and setscrew on vessel and tighten.
12. Rinse outside of sealed vessel in acetone and allow to air-dry.
13. Insert sealed vessel into quartz envelope and evacuate on vacuum extraction line.
14. Bake sealed vessel in evacuated quartz envelope at 1050 °C for 50 minutes. After baking, allow quartz envelope and vessel to cool to room temperature (30 minutes).

#### C) Collection of CO<sub>2</sub> (see Fig. 5)

1. Preheat tube oven to 350 °C (to decompose traces of Ni(CO)<sub>4</sub> produced in the vessels; Brenninkmeijer & Mook, 1981) at least one hour in advance to permit thermal stabilization. Note that the Ni-trap should be cleaned in 10% nitric acid routinely to prevent excess build-up of Ni and carbon.
2. Establish a vacuum downstream of valve #1 (i.e. open valves #2, #3, and #4).
3. Place a cooled pyrolysis vessel inside the sample shaft of the puncturing device. Check that the puncturing needle is withdrawn to the correct height and that o-rings are clean. Replace securing head and puncturing hilt and screw tightly to the sample shaft.

4. Attach the puncturing device to the vacuum line; open valve #1 to evacuate the puncturing device. Pump on the entire line for several minutes until vacuum is obtained.
5. Place liquid nitrogen dewar on bottom of u-trap. Close valve #1. Carefully screw down the puncturing hilt (resistance will indicate that the pyrolysis vessel has been pierced). Slowly raise the hilt to remove the needle from the pyrolysis vessel and allow the gases to escape onto the heated portion of the line.
6. Allow the gases to remain in the heated portion of the line for 2 minutes. Close valve #2. Slowly open valve #1 and allow the CO<sub>2</sub> gas to condense in u-trap.
7. Record change in pressure from the presence of non-condensable gases (mainly argon and CO).
8. Raise liquid nitrogen dewar halfway up u-trap; wait several minutes and then raise to about 3 cm from top of u-trap.
9. Bleed off non-condensable gas by opening valve #2 slowly. Pump on the trapped CO<sub>2</sub> to remove non-condensable gases and to ensure complete transfer from punctured sample vessel. When the initial (full vacuum) reading on the pressure gauge is restored, close valves #1 and #2.
10. Replace liquid nitrogen dewar with ethanol/dry ice dewar on u-trap to vaporize the CO<sub>2</sub>. Record the new reading on the pressure gauge.
11. Place a liquid nitrogen dewar on the bottom of sample breakseal and close valve #3 to isolate the line from the vacuum pump. Slowly open valve #2 and allow the CO<sub>2</sub> to condense in the sample breakseal. Raise liquid nitrogen dewar over condensed CO<sub>2</sub>.
12. After a few minutes, during which the pressure gauge should have returned to near its full-vacuum reading, raise liquid nitrogen dewar. Open valve #3 to pump off any residual non-condensable gases that may have remained in the solid CO<sub>2</sub>.
13. Flame off sample breakseal. Ensure that the sample vessel is correctly labeled and remove it for analysis by mass spectrometry.
14. Close valves #1 and #2. Heat for 2–3 minutes and check for an increase in pressure. A change may indicate the presence of water vapour suggesting that the pyrolysis reaction was incomplete. Open valve #2 to check vacuum recovery. A substantial fall in the vacuum also suggests residual water. Replace sample breakseal, change pyrolysis vessel in puncturing device, and re-evacuate line.

New pyrolysis vessels require conditioning before analysis. Standards should be loaded, pyrolysed and the resulting gas released for at least two runs before CO<sub>2</sub> is collected for analysis. Repeated runs with water and cellulose standards should then be undertaken until consistent reproducibility is obtained and until accurate calibration of results can be developed. After extended periods of non-use, standards should be run in each vessel to re-establish the catalytic activity of the nickel. A water or cellulose standard should be routinely run with each sample batch, rotating the vessel in which the standard is loaded to monitor vessel performance. Analytical uncertainties of water, lake sediment and terrestrial cellulose are generally  $\pm 0.5$  to 1.0‰.

### Key criteria for paleohydrologic reconstruction

Two fundamental assumptions in the interpretation of carbon and oxygen isotope data from lake sediment cellulose are 1) the fine-grained cellulose fraction in offshore lake sediments is derived from aquatic plants/algae and 2) the oxygen isotope fractionation between ambient water and aquatic cellulose is known (or constant), allowing for reconstruction of lake water  $\delta^{18}\text{O}$  history from the sediment record. The validity of these assumptions is briefly discussed below.

#### *Origin of lake sediment cellulose*

Incorporation of terrestrial cellulose in fine-grained offshore lake sediment would appear to be a primary complicating factor, yet  $\delta^{18}\text{O}_{\text{cell}}$  records obtained from lakes in many hydrological and ecological settings seem to support a dominantly aquatic origin (Edwards & McAndrews, 1989; Duthie et al., 1996; Edwards et al., 1996; Padden, 1996; Beuning et al., 1997; Buhay & Betcher, 1998; Abbott et al., 2000; Wolfe et al., 2000b). Less favourable sites include lakes characterized by low aquatic productivity relative to allochthonous organic matter input and sediment that undergoes substantial post-depositional loss of organic matter (e.g., Sauer et al., 2000). Physical removal of coarse organic debris by sieving is evidently a key step in sample preparation, as this fraction may be derived in part from terrestrial sources (e.g., Meyers, 1997). Source interpretation of cellulose may be additionally constrained by supplementary isotopic and elemental data. For instance, low C/N ratios ( $< 10$ ) commonly indicate aquatic-derived organic matter reflective of the higher mean protein content of microplankton (mean  $\sim 35\%$ ) as compared to terrestrial plants (only 1–8%) (Tyson, 1995; Meyers & Lallier-Vergès, 1999). However, this aquatic-origin “boundary” of C/N  $< 10$  is not exclusive (see Tyson, 1995). Even in sediments with C/N ratios ranging from 10 to  $> 15$ , oxygen isotope analyses of the cellulose fraction appear to predominantly reflect lake water  $\delta^{18}\text{O}$  based on close correspondence between inferred lake water oxygen isotope composition from surface sediment cellulose and the isotopic composition of the overlying lake water (Duthie et al., 1996; Beuning et al., 1997; Abbott et al., 2000) and other supporting evidence (Wolfe et al., 1999, 2000b). This consistency may perhaps be due to preferential preservation of aquatic cellulose as compared to other organic compounds due to rapid sedimentation and burial (Edwards, 1993). Alternatively, high C/N ratios in aquatic organic matter have been documented for cellulose-producing green algae such as *Botryococcus* (C/N  $\approx 36$ ) (Street-Perrott et al., 1997). In other cases, high C/N ratios may result from lake waters that are nitrogen deficient (Talbot & Lærdal, 2000) or may be due to diagenetic effects on organic matter that originally derived from aquatic sources (Meyers & Ishiwatari, 1993; Tyson, 1995). Hydrogen indices can serve as an additional test of an aquatic source for sedimentary organic matter (Talbot & Livingstone, 1989; Talbot & Lærdal, 2000).

Parallel time-series trends between bulk organic carbon isotope ( $\delta^{13}\text{C}_{\text{org}}$ ) and cellulose carbon isotope ( $\delta^{13}\text{C}_{\text{cell}}$ ) profiles may indicate carbon uptake from the same source (i.e. DIC) and can provide further indirect support for an aquatic origin of cellulose. Conversely, stratigraphic variation in the isotopic difference between  $\delta^{13}\text{C}_{\text{org}}$  and  $\delta^{13}\text{C}_{\text{cell}}$  may be due to variable terrestrial contribution (Wolfe et al., 1996). However, these empirical relationships can be complicated by the lower sensitivity of bulk organic matter to fluctuations in

DIC  $\delta^{13}\text{C}$ , as well as temporal changes in bulk organic matter preservation (Edwards & McAndrews, 1989).

#### *Cellulose-water oxygen isotope fractionation*

Both laboratory and field studies have been conducted to investigate the relationship between the oxygen isotope composition of aquatic cellulose and the oxygen isotope composition of the water in which the plants or algae grew (Epstein et al., 1977; DeNiro & Epstein, 1981; Sternberg et al., 1984, 1986; Edwards et al., 1985; Yakir & DeNiro, 1990; Sauer et al., 2000; Abbott et al., 2000; Beuning & Anderson, 2000). Many of these data indicate that the oxygen isotope fractionation between cellulose and water is nearly constant, being independent of the oxygen isotope composition of  $\text{CO}_2$ , the plant species, the water temperature, and the photosynthetic mode of the organism. Reported cellulose-water oxygen isotope fractionation factors are mostly within the range of 1.025 to 1.029 for most aquatic plant/algae species in which both the water and cellulose have been measured directly (Table I).

Two primary models have been proposed to account for the constant cellulose-water oxygen isotope enrichment factor (see reviews by Sternberg, 1989b and Yakir, 1992). Epstein et al. (1977) suggested that during cellulose synthesis, oxygen is incorporated from dissolved  $\text{CO}_2$  and  $\text{H}_2\text{O}$  (which are in isotopic equilibrium) in a 1 : 1 ratio. As a result, 2/3 of the oxygen in cellulose is derived directly from dissolved  $\text{CO}_2$  enriched in  $^{18}\text{O}$  relative to the plant water by about 41‰, according to an oxygen isotope fractionation factor of 1.0412 between  $\text{CO}_2$  and  $\text{H}_2\text{O}$  at 25 °C (thus  $2/3 \times 41 \approx 27\%$ ). As discussed by Yakir (1992), however, there are several difficulties with this theory including: 1) oxygen incorporated from water is lost during subsequent metabolic reactions, 2) the isotopic relationship is consistent in plants that do not consume  $\text{CO}_2$  (heterotrophic metabolism), and 3) the isotopic effect associated with  $\text{CO}_2$  equilibration with water is highly sensitive to temperature, yet no clear temperature-dependent isotope effect has been demonstrated between water and cellulose.

The second alternative model favoured by most workers suggests that the oxygen isotope composition of plant cellulose is determined by the carbonyl hydration reaction which occurs during cellulose synthesis (DeNiro & Epstein, 1981). According to this theory, carbonyl oxygen atoms exchange with water in an equilibrium reaction that is apparently insensitive to temperature. Experiments analyzing oxygen isotope exchange between the carbonyl oxygen of acetone and water across a temperature range of 15 to 35 °C resulted in fractionation factors ranging from 1.025 to 1.030, similar to those observed between cellulose and water (Sternberg & DeNiro, 1983).

Prior to 1998, the majority of field studies examining cellulose-water oxygen isotope fractionation were conducted in temperate aquatic environments (Table I). Recent calibration work by Beuning & Anderson (2000) from samples collected in tropical East African lakes (also shown in Table I) suggests a lower mean fractionation factor ( $1.025 \pm 0.003$ ) than samples from temperate freshwater environments ( $1.027 \pm 0.003$ ). This difference could result from sampling of  $^{18}\text{O}$ -enriched water at the end of the dry season, but only newly emerged leaves were used in the analyses. In addition, samples from large lake systems with minimal seasonal fluctuations in lake water  $\delta^{18}\text{O}$  also showed reduced cellulose-water

Table I. Published values for  $\alpha_{\text{cell-water}}$ . Marine field values from DeNiro & Epstein (1981) represent ranges based on winter and summer  $\delta^{18}\text{O}$  values for the water. Values from Sternberg et al. (1984) were estimated from Figure 1. Values from Sauer et al. (2000) are adjusted assuming a constant  $\alpha_{\text{cell-water}}$  of 1.028 in aquarium studies of cultured mosses under controlled conditions.

Field (F) or Laboratory (L)	T (°C)	$\alpha_{\text{cell-water}}$	Reference
<b>Freshwater vascular plants</b>			
<i>Ceratopteris</i> sp.	L 20	1.0292	DeNiro & Epstein (1981)
<i>Cryptocoryne</i> sp.	L 25	1.0289	
<i>Hygrophila polysperma</i>	L 20	1.0266	
	L 25	1.0273	
<i>Ludwigia natans</i>	L 15	1.0262	
	L 20	1.0258	
	L 25	1.0265	
<i>Nymphoides aquaticum</i> (roots)	L 20	1.0289	
<i>Nymphoides aquaticum</i> (submerged leaves)	L 20	1.0285	
<i>Nymphoides aquaticum</i> (floating leaves)	L 20	1.0289	
<i>Synnema triflorum</i>	L 15	1.0267	
	L 20	1.0262	
<i>Vallisneria spiralis</i>	L 25	1.0289	
<i>Isoetes howellii</i>	L	1.0290	Sternberg et al. (1984)
<i>Ranunculus aquatilis</i>	L	1.0260	
<i>Chara contraria</i>	L	1.0280	
<i>Vallisneria spiralis</i>	L 25	1.0285	
<i>Ludwigia natans</i>	L 15	1.0255	
	L 25	1.0265	
<i>Ceratopteris</i> sp.	L 20	1.0255	
<i>Hygrophila polysperma</i>	L 20	1.0290	
	25	1.0255	
<i>Synnema triflorum</i>	L 15	1.0260	
	20	1.0255	
<i>Isoetes bolanderi</i>	F	1.0270	
<i>Chara contraria</i>	F	1.0265	
<i>Chara longipedunculata</i>	F	1.0240	
<i>Fontinalis antipyretica</i>	F	1.0260	
<i>Eleocharis acicularis</i>	L	1.0300	
<i>Ranunculus aquatilis</i>	F	1.0286	
<i>Sphagnum</i> sp.	F	1.0253	Aucour et al. (1993)
<i>Nymphaea</i> sp.	F	1.0250	Aucour et al. (1996)
green algae	F	1.0294	Abbott et al. (2000)
<i>Myriophyllum</i>	F	1.0287	
submerged aquatic moss	F	1.0287	Sauer et al. (2000)
	F	1.0263	
	F	1.0278	
	F	1.0285	
	F	1.0249	
	F	1.0265	

Table I. Published values for  $\alpha_{\text{cell-water}}$ . Marine field values from DeNiro & Epstein (1981) represent ranges based on winter and summer  $\delta^{18}\text{O}$  values for the water. Values from Sternberg et al. (1984) were estimated from Figure 1. Values from Sauer et al. (2000) are adjusted assuming a constant  $\alpha_{\text{cell-water}}$  of 1.028 in aquarium studies of cultured mosses under controlled conditions (continued).

Field (F) or Laboratory (L)	T (°C)	$\alpha_{\text{cell-water}}$	Reference
	F	1.0277	
	F	1.0290	
	F	1.0236	
	F	1.0280	
	F	1.0250	
	F	1.0293	
	F	1.0274	
	F	1.0276	
	F	1.0292	
	F	1.0286	
	F	1.0292	
	F	1.0261	
	F	1.0253	
	F	1.0287	
	F	1.0269	
	F	1.0276	
	F	1.0334	
	F	1.0238	
	F	1.0284	
	F	1.0287	
	F	1.0297	
	F	1.0298	
	F	1.0299	
	F	1.0313	
	F	1.0319	
	F	1.0300	
	F	1.0312	
	F	1.0316	
	F	1.0306	
	F	1.0301	
<i>Eicchornia</i>	F	1.0260	Beuning & Anderson (2000)
<i>Eicchornia</i> - leaves	F	1.0255	
<i>Ceratophyllum demersum</i>	F	1.0234	
KRB 105 (Unknown)	F	1.0225	
<i>Najas</i> cf. <i>horrida</i>	F	1.0268	
KRB 112 (Unknown)	F	1.0247	
<i>Ceratophyllum</i> sp.	F	1.0248	
<i>Lemna trisulca</i>	F	1.0256	
<i>Botryococcus braunii</i>	L	1.0249	
Characeae (cf. <i>Chara</i> )	F	1.0246	
<i>Nymphaea</i> -leaves only	F	1.0253	
<i>Nymphaea</i> -flower	F	1.0247	

Table I. Published values for  $\alpha_{\text{cell-water}}$ . Marine field values from DeNiro & Epstein (1981) represent ranges based on winter and summer  $\delta^{18}\text{O}$  values for the water. Values from Sternberg et al. (1984) were estimated from Figure 1. Values from Sauer et al. (2000) are adjusted assuming a constant  $\alpha_{\text{cell-water}}$  of 1.028 in aquarium studies of cultured mosses under controlled conditions (continued).

Field (F) or Laboratory (L)	T (°C)	$\alpha_{\text{cell-water}}$	Reference
<i>Nymphaea</i> -stem only	F	1.0251	
<i>Nymphaea</i> -leaves & stem	F	1.0253	
<i>Najas</i>	F	1.0221	
Characeae (cf. <i>Chara</i> )	F	1.0270	
KRB 109 - leaves	F	1.0264	
<b>Marine aquatic plants and algae</b>			
Turtle grass	F	1.0272	Epstein et al. (1977)
<i>Phyllospadix</i>	F	1.0280	
Eurasian water milfoil	F	1.0276	
<i>Thalassia testudinum</i>	F	1.0276	DeNiro & Epstein (1981)
<i>Macrocystis</i> sp.	F	1.0293–1.0280	
<i>Laminaria</i> sp.	F	1.0283–1.0272	
<i>Gelidium robustum</i>	F	1.0291–1.0285	
<i>Macrocystis</i> sp.	F	1.0270–1.0264	
<i>Ulva</i> sp.	F	1.0254–1.0248	
<i>Phyllospadix torreyi</i>	F	1.0290–1.0284	
<i>Zostera marina</i>	F	1.0285–1.0279	
<i>Ascophyllum nodosum</i>	F	1.0299–1.0263	
<i>Chondria tenuissima</i>	F	1.0290–1.0254	
<i>Chondrus crispus</i>	F	1.0304–1.0268	
<i>Fucus vesiculosus</i>	F	1.0306–1.0270	
<i>Laminaria agardhii</i>	F	1.0303–1.0267	
<i>Polysiphonia nigrescens</i>	F	1.0304–1.0268	
<i>Ulva lactuca</i>	F	1.0290–1.0254	
<i>Thalassia</i> sp.	F	1.0316–1.0280	

isotopic fractionation. While these results suggest a possible small temperature-dependent effect on the fractionation factor between cellulose and water, clearly, additional field studies, particularly in tropical environments, are required to clarify this discrepancy (Beuning & Anderson, 2000).

Assuming a constant oxygen isotope fractionation between cellulose and water,  $\delta^{18}\text{O}$  analysis of the cellulose component of surface sediment samples may be consistent with contemporary water and plant samples, as has been shown for Lago Taypi Chaka Kkota, Bolivia (e.g. Abbott et al., 2000). This lake is characterized by suppressed seasonal isotopic variability due to perennially through-flow conditions and thus single-episode sampling of sediments, plants and lake water, which frequently forms the basis for “calibration”, produced coherent results. Indeed, caution must be exercised in evaluating water and sediment data of strongly differing temporal representation. For example, lakes near boreal treeline in central Siberia are susceptible to strong seasonal isotopic variation. As a result, single-episode water and surface sediment sampling demonstrated that the hydrologic conditions

inferred from the surface sediments at most sites were systematically offset from those prevailing at the time of sampling due to the influence of snowmelt on lake water isotopic composition (see Wolfe & Edwards, 1997).

## Applications

### *Holocene water and carbon balance record of treeline lakes, central Canada*

Reconstruction of lake water  $\delta^{18}\text{O}$  ( $\delta^{18}\text{O}_{\text{lw}}$ ) histories from analysis of fine grained cellulose at small treeline lakes in central Canada has provided key insight into shifting water balance and its influence on watershed carbon cycling during the Holocene (MacDonald et al., 1993; Wolfe et al., 1996). In this region, terrestrial vegetation change following local deglaciation around 9000  $^{14}\text{C}$  yr B.P. was characterized by the advance and retreat of boreal treeline, in response to shifts in the mean summer position of the Arctic frontal zone (Dyke & Prest, 1987; MacDonald et al., 1993). Abrupt changes from dwarf shrub tundra to *Picea mariana* forest-tundra occurred at about 5000  $^{14}\text{C}$  yr B.P. (Fig. 6), as the frontal zone moved northward. Minor local fluctuations in treeline position or forest density occurred during the subsequent 2000 years, followed by return to the modern dwarf shrub tundra vegetation after 3000  $^{14}\text{C}$  yr B.P.

Cellulose isotope records from Queen's Lake (MacDonald et al., 1993), located 25 km north of the forest-tundra zone, and Toronto Lake (Wolfe et al., 1996), situated at the transition between forest-tundra and tundra, show shifts to lower  $\delta^{18}\text{O}_{\text{lw}}$  values during the mid-Holocene (Fig. 6), reflecting decreased evaporative  $^{18}\text{O}$ -enrichment with a northward shift in the Arctic front. An isotope mass-balance model, based primarily on the Queen's Lake  $\delta^{18}\text{O}_{\text{lw}}$  record, suggests relative humidity may have increased by 10–15% overall during this interval (Edwards et al., 1996). High-resolution analysis of the Toronto Lake sediments indicates century-scale correspondence between increasingly moist conditions and forest expansion (Fig. 6; Wolfe et al., 1996). The hydrologic sensitivity of Toronto Lake may be largely a function of the lake's complex drainage basin. Under present climatic conditions, the two subcatchments providing inflow to Toronto Lake have markedly different hydrological budgets. The larger of the two subcatchments (ca. 2100 ha) yields continuous outflow during the thaw season, whereas the smaller subcatchment (ca. 650 ha) apparently discharges only intermittently following spring melt. Abrupt changes in the size of the contributing drainage basin area, in response to changes in moisture conditions in the past, appear to have strongly influenced lake water residence time and degree of evaporative enrichment at Toronto Lake. In contrast, Queen's Lake drains a considerably smaller catchment than Toronto Lake and thus contains a  $\delta^{18}\text{O}_{\text{lw}}$  record that is largely dependent on the hydrologic sensitivity of this single lake, which has apparently remained closed for most of the Holocene.

The forest-tundra interval at Queen's Lake and Toronto Lake was also marked by changes in carbon balance. Increased organic content (i.e., high loss-on-ignition) and diatom valve concentration clearly indicate that lake productivity increased during this interval (MacDonald et al., 1993; Pienitz et al., 1999). Increasing  $\delta^{13}\text{C}_{\text{cell}}$  values during the mid-Holocene are likely caused by this elevated lake productivity, through photosynthetically-driven  $^{13}\text{C}$ -enrichment of dissolved inorganic carbon (DIC) and decreased cellulose-DIC isotope fractionation. However, at various times during the Holocene, contemporaneous

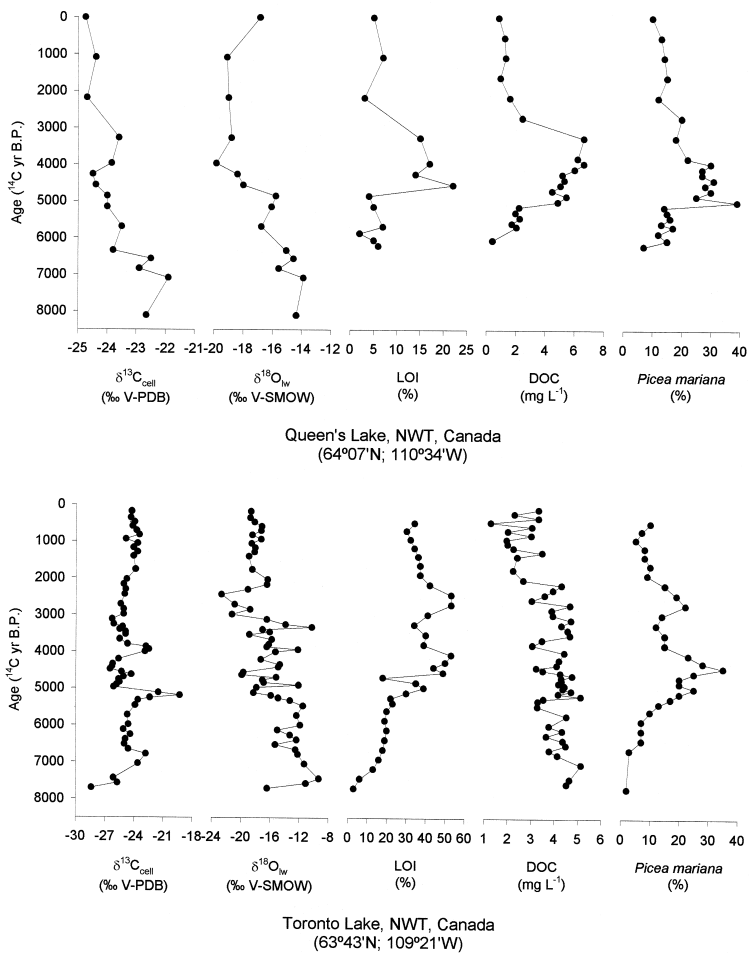


Figure 6. Stratigraphic profiles of cellulose carbon isotope composition ( $\delta^{13}\text{C}_{\text{cell}}$ ), cellulose-inferred lake water  $\delta^{18}\text{O}$  composition ( $\delta^{18}\text{O}_{\text{lw}}$ ) calculated using a cellulose-water fractionation factor of 1.028, loss-on-ignition (LOI), diatom-inferred dissolved organic carbon concentration (DOC), and *Picea mariana* pollen concentration for Queen's Lake and Toronto Lake, Northwest Territories, Canada (from MacDonald et al., 1993; Wolfe et al., 1996; Pienitz et al., 1999).

rapid lake water through-flow and supply of soil-derived  $^{13}\text{C}$ -depleted  $\text{CO}_{2(\text{aq})}$  have probably played an important role in offsetting and, in some cases, completely masking these isotopic enrichment signatures (i.e., compare LOI with  $\delta^{13}\text{C}_{\text{cell}}$  at 3500  $^{14}\text{C}$  yr B.P. at Queen's Lake; at 5100, 4500, 4000, 2700  $^{14}\text{C}$  yr B.P. at Toronto Lake; Fig. 6). Increasingly moist conditions combined with developing soils and forest-tundra vegetation during the mid-Holocene are also reflected by the high diatom-inferred dissolved organic carbon (DOC) concentrations indicating increased humus input (Fig. 6; Pienitz et al., 1999). At Toronto Lake, the "noisier" DOC record is likely related to the hydrologic variability at this

site, whereas the apparent high values during the early Holocene are considered unreliable due to a “no-analog” application of the diatom-based transfer function (see Pienitz et al., 1999).

*Postglacial record of the isotopic composition of precipitation in the Great Lakes region*

In lake basins for which evaporation is a small component of the water balance (and where the primary inputs of water derive from precipitation or inflow that has experienced little evaporative evolution), sediment-inferred lake water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  histories may reflect the mean annual isotope composition of precipitation ( $\delta^{18}\text{O}_p$ ;  $\delta^2\text{H}_p$ ). This parameter is frequently used as a tracer of past temperature change as well as evidence for changes in moisture sources, seasonal distribution of precipitation, and other aspects of air mass circulation.

In the Great Lakes region of North America, recent oxygen and hydrogen isotope studies of lake sediment cellulose and lake sediment kerogen, respectively, in addition to  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  analysis of fossil wood cellulose, have resulted in a largely consistent pattern of shifting isotopic composition of post-glacial precipitation. The initial reconstruction was based on isotopic evaluation of fossil wood cellulose preserved in a kettle-fill sequence near Brampton, Ontario (Edwards & Fritz, 1986). A semi-empirical model was used to separate humidity-dependent isotopic enrichment of leaf water during evapotranspiration from the primary isotopic signature of water taken up by the trees, presumably reflecting the local mean annual isotopic composition of precipitation. Calibration of the model using modern trees permitted quantitative reconstruction of both  $\delta^{18}\text{O}_p$  as well as relative humidity during the growth season for the past 11,500  $^{14}\text{C}$  years (Fig. 7a).

Oxygen isotope analysis of fine-grained cellulose in sediments underlying Hamilton Harbour, a bay at the western end of Lake Ontario, have recently verified and further supplemented the later part of the regional  $\delta^{18}\text{O}_p$  record from about 8000  $^{14}\text{C}$  yr B.P. to present (Duthie et al., 1996). Comparison of the Hamilton Harbour cellulose-inferred lake water  $\delta^{18}\text{O}$  ( $\delta^{18}\text{O}_{\text{lw}}$ ) record with the reconstructed precipitation  $\delta^{18}\text{O}$  history from the Brampton site shows that lake water  $\delta^{18}\text{O}$  was primarily controlled by the changing isotopic composition of precipitation falling in the region (Fig. 7b). The only strong evidence of local hydrologic overprinting is the marked positive offset of  $\delta^{18}\text{O}_{\text{lw}}$  at the base of the profile, likely due to evaporative enrichment when the harbour was isolated from Lake Ontario. Rising water level in Lake Ontario and confluence of harbour and lake water is reflected by reduced evaporative enrichment and subsequent decrease in Hamilton Harbour  $\delta^{18}\text{O}_{\text{lw}}$  at about 7000  $^{14}\text{C}$  yr B.P. This major hydrologic event is also clearly demarcated in the diatom and cellulose carbon isotope records (Duthie et al., 1996; Wolfe et al., 2000c).

At Austin Lake, southwestern Michigan,  $\delta^2\text{H}$  analysis of sediment kerogen has been used to reconstruct late Glacial and Holocene lake water  $\delta^2\text{H}$  ( $\delta^2\text{H}_{\text{lw}}$ ) history (Fig. 7c; Krishnamurthy et al., 1995). The kerogen-inferred  $\delta^2\text{H}_{\text{lw}}$  record was derived from extending the empirical relationship between surface-sediment kerogen  $\delta^2\text{H}$  and lake-recharged groundwater  $\delta^2\text{H}$  down-core. The relationship closely resembles the hydrogen isotope fractionation between non-exchangeable hydrogen in cellulose and ambient water reported by Yapp & Epstein (1982). Notably, groundwater  $\delta^2\text{H}$  in this region is similar to local

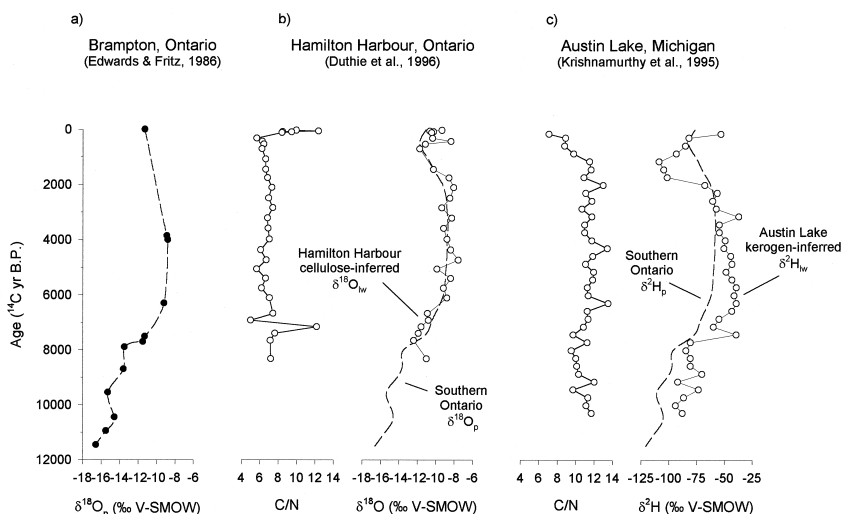


Figure 7. a) Mean annual oxygen isotope composition of precipitation ( $\delta^{18}\text{O}_p$ ) history derived from isotope analysis of fossil wood cellulose near Brampton, Ontario. b) Hamilton Harbour bulk organic C/N ratio and cellulose-inferred lake water  $\delta^{18}\text{O}$  ( $\delta^{18}\text{O}_{\text{Iw}}$ ) profiles. Also shown is the  $\delta^{18}\text{O}_p$  record for southern Ontario from a) modified slightly during the late Holocene to account for the newer data from Hamilton Harbour. Low C/N ratios indicate that the organic matter is derived predominantly from aquatic sources (cf. Meyers & Lallier-Verges, 1999). The abrupt increase in C/N ratios at the top of the core is likely related to watershed deforestation and increased terrestrial input of organic matter, although this does not seem to have strongly influenced the cellulose  $\delta^{18}\text{O}$  record. Notably,  $\delta^{18}\text{O}_{\text{Iw}}$  (calculated using a cellulose-water fractionation factor of  $1.028 \pm 0.001$ ) in the uppermost sediment sample ( $-9.4 \pm 1.0\text{‰}$ ) is similar to surface water isotope data sampled in May, 1994 ( $-8.3$  to  $-7.8\text{‰}$ ; Harvey et al., 1997). c) Austin Lake bulk organic C/N ratio and kerogen-inferred lake water  $\delta^2\text{H}$  ( $\delta^2\text{H}_{\text{Iw}}$ ) history calculated using a kerogen-water fractionation factor of 0.9725. The southern Ontario  $\delta^2\text{H}_p$  record is also shown, calculated from b) using the local meteoric water line for Simcoe ( $\delta^2\text{H} = 7.92 \delta^{18}\text{O} + 10.53$ ) reported in Fritz et al. (1987). The “latitude effect” is likely responsible for the positive offset of Austin Lake  $\delta^2\text{H}_{\text{Iw}}$  from Brampton  $\delta^2\text{H}_p$  for most of the profile (see Fig. 1; also Rozanski et al., 1993). Further work is needed to evaluate the large  $\delta^2\text{H}_{\text{Iw}}$  oscillation during the late Holocene.

$\delta^2\text{H}_p$  so as a first-order approximation, Krishnamurthy et al. (1995) assumed the kerogen-inferred  $\delta^2\text{H}_{\text{Iw}}$  record was primarily a reflection of the  $\delta^2\text{H}_p$  history. This interpretation is strongly supported by correlative trends in the records from Brampton and Hamilton Harbour (Fig. 7a, b). The lower parts of these profiles, in particular, show striking agreement and are characterized by low values during the late-Glacial and early Holocene rising to maximum values in the mid-Holocene.

The record of post-glacial isotopic composition of precipitation in the Great Lakes region is likely a reflection of both changes in temperature and air mass circulation (Edwards & Fritz, 1986; Krishnamurthy et al., 1995; Edwards et al., 1996). Increasing  $\delta^{18}\text{O}_p$  and  $\delta^2\text{H}_p$  values from the post-Glacial to the mid-Holocene appear to result from progressive post-glacial warming as influence of Arctic air diminished and warm, moist,  $^{18}\text{O}$ -enriched air masses from the Gulf of Mexico became increasingly prevalent. Superimposed on this meridional fluctuation in atmospheric circulation is a shorter-term episode of enhanced zonal circulation between 7500 and 6000  $^{14}\text{C}$  yr B.P. that accounts for a lag between rising

temperature and humidity (Bryson & Wendland, 1967; Edwards & Fritz, 1986; Dean et al., 1996; Edwards et al., 1996). Increased penetration of warm, dry Pacific air into the continental interior during the summer months likely resulted in increased lake water evaporation which may partly account for the abrupt  $^2\text{H}$ -enrichment at about 7000  $^{14}\text{C}$  yr B.P. in the Austin Lake record. The end of the warm mid-Holocene is clearly shown by the shift to lower Austin Lake  $\delta^2\text{H}_{\text{lw}}$  and Hamilton Harbour  $\delta^{18}\text{O}_{\text{lw}}$  values at about 2000  $^{14}\text{C}$  yr B.P., reflecting decreased influence of air masses from the Gulf of Mexico, and is consistent with other paleoclimatic records from southern Ontario (see Edwards et al., 1996). A subsequent return to warmer conditions is suggested by increasing Austin Lake  $\delta^2\text{H}_{\text{lw}}$  and Hamilton Harbour  $\delta^{18}\text{O}_{\text{lw}}$  values after 1000  $^{14}\text{C}$  yr B.P.

### **Future research directions**

Isotope analysis of lake sediment cellulose has undergone rapid growth over the last decade as a useful approach for paleohydrologic and paleoclimatic reconstruction. The need remains, however, for further research into key areas of analysis and application to refine this technique and to improve upon its contribution to paleolimnology. Specific areas to be addressed are outlined below.

#### *Sample preparation and analytical techniques*

Oxygen isotope analysis of lake sediment cellulose is currently a labour-intensive process, requiring off-line  $\text{CO}_2$  gas purification prior to mass spectrometric analysis. However, recent development of successful on-line techniques (i.e., CF-IRMS) for  $\delta^{18}\text{O}$  analysis of organic compounds (e.g., Farquhar et al., 1997; Saurer et al., 1998) suggests that similar methods may be utilized to expedite lake sediment cellulose  $\delta^{18}\text{O}$  analysis. Preliminary analyses using CF-IRMS at the EIL suggest that development of a viable technique requires the removal of refractory material from the cellulose residue. Modifications to the sample preparation technique are currently in development including flotation with sodium polytungstate to separate cellulose from mineral detritus. Improvements in analytical capabilities will increase the cost-effectiveness of studies and may allow for very small samples to be analyzed including cellulose derived from identifiable aquatic remains in sediment cores. In addition, multiple analysis of several individual samples will warrant more comprehensive evaluation of differences in sample reproducibility (from site to site and also stratigraphically), which may also potentially reveal information characteristic of a lake's hydrologic setting and variability (see Wolfe et al., 2000b).

#### *Investigation of cellulose-water fractionation*

Recent work by Beuning & Anderson (2000) clearly points to the need for more studies examining the oxygen isotope relationship between aquatic cellulose and ambient water under varied conditions. While applications of a constant fractionation factor in the past have proven to be a useful first-order approximation, this critical assumption requires further testing. Additional laboratory-based studies are in progress, where the effects of

key parameters (e.g. temperature, species) on the oxygen isotope composition of important cellulose-producing aquatic plants may be re-evaluated (Beuning, unpublished data). Greater understanding of the cellulose-water  $\delta^{18}\text{O}$  relationship in natural settings would also benefit from comprehensive field surveys involving multiple lake water samples to characterize intra-annual lake water isotopic composition and variability, sediment trap collections to measure aquatic cellulose  $\delta^{18}\text{O}$  *in situ*, and corresponding surface-sediment samples for lake sediment cellulose  $\delta^{18}\text{O}$  determination.

#### *Characterization of sediment cellulose origins*

Organic petrography needs to be explored as a potentially useful complementary tool for evaluating the significance of terrestrial cellulose contributions to the fine-grained sediment component of lake sediments. This and other sediment identification techniques (e.g., scanning electron microscopy) may also prove useful for determining if the lake sediment cellulose is derived from varied aquatic sources, with species-specific oxygen isotope fractionation factors.

#### *Quantitative applications using multiple isotopic archives*

Interpretation of cellulose-inferred lake water  $\delta^{18}\text{O}$  records would benefit from coupled estimation of lake water  $\delta^2\text{H}$  in order to clearly decipher shifting isotopic composition of precipitation from changes in water balance (see Fig. 1). Exploratory paleolimnological reconstructions at Austin Lake demonstrate that further studies on the hydrogen isotope composition of kerogen and its isotopic relationship with ambient water is an especially promising new research direction (Krishnamurthy et al., 1995; also see Fig. 7). "Mapping" temporal shifts in lake water isotopic composition in two-dimensional  $\delta^{18}\text{O}$ - $\delta^2\text{H}$  space would also provide a more solid foundation for quantitative reconstructions. For example, stratigraphic changes that occur along a well-defined local evaporation line may be used to determine shifting percentages of lake water evaporative loss, as in contemporary hydrologic investigations (e.g., Gibson et al., 1993).

Coupled carbon and oxygen isotope analyses of carbonate and organic fractions should also be further exploited as a paleolimnological approach as these studies provide strong potential for quantitative reconstruction of past lake water temperature (Padden, 1996; Padden et al., 1996; Wolfe et al., 2000a).

### **Summary**

Systematic variations occur in the isotopic composition of water as it passes through the hydrologic cycle due to fractionation effects that separate the common, light stable isotope from the rare, heavy isotope. The isotopic composition of lake water captures a time-integrated reflection of these processes, which are strongly influenced by climatic and hydrologic factors. Aquatic cellulose readily incorporates the oxygen isotope composition of lake water and this signature is preserved in lake sediment stratigraphic records. Hydrologic as well as lake nutrient balance information may additionally be contained in profiles of lake sediment cellulose carbon isotope composition. Although case studies presented

here focus on qualitative interpretation of the isotope profiles, results from both cellulose carbon and oxygen isotope analyses also offer the potential for quantitative recovery of paleohydrologic and paleoclimatic information that may not necessarily be readily attainable from other conventional sources of proxy data (e.g., Edwards & McAndrews, 1989; Edwards et al., 1996; Wolfe et al., 2000a, c). We emphasize, however, that interpretation is most effectively constrained within the context of a multidisciplinary approach (e.g., Abbott et al., 2000). High priority is currently focused on simplification of the analytical procedure in order to foster more routine incorporation of cellulose-isotope analyses in paleolimnological investigations.

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