Paper:
http://dx.doi.org/10.1016/j.quascirev.2018.05.010
The Holocene isotopic record of aquatic cellulose from Lake Antu Sinijärv, Estonia: Influence of changing climate and organic-matter sources

F. Alayne Street-Perrott a,*, Jonathan A. Holmes b, Iain Robertson a, Katherine J. Ficken a, Tiitu Koff c, Neil J. Loader a, Jim D. Marshall d, Tõnu Martma e

* Department of Geography, College of Science, Swansea University, Swansea, SA2 8PP, UK
b Institute of Ecology, Tallinn University, Lus-Sadama 5, 10220, Tallinn, Estonia
c Environmental Change Research Centre, Department of Geography, University College London, Gower Street, London, WC1E 6BT, UK
d Department of Earth, Ocean and Ecological Sciences, University of Liverpool, Liverpool, L69 3GP, UK
e Department of Geology, Tallinn University of Technology, Ehitajate tee 5, 10120, Tallinn, Estonia

A R T I C L E  I N F O

Article history:
Received 30 September 2017
Received in revised form 25 April 2018
Accepted 9 May 2018

A B S T R A C T

The well characterized oxygen-isotopic fractionation during cellulose biosynthesis has been utilised by numerous studies of stable isotopes in fine-grained aquatic cellulose. We measured the δ 13C cellulose and δ 18O cellulose values of bulk cellulose and moss fragments from an ~11.4ka-long core obtained from a shallow, productive, spring-fed, hardwater lake, Antu Sinijärv, Estonia (59°3.8′N; 26°14.5′E; 94.6 m a.s.l.; maximum depth 7.3 m), in order to reconstruct regional Holocene climate and lake-basin evolution. Isotopically, the modern waterbody is a well-behaved, open, hydrological system with negligible evaporative effects. Cellulose-isotope records were compared with down-core measurements of loss-on-ignition (LOI), carbonate and mineral contents, total organic carbon (TOC), total nitrogen (TN), C/N ratio, δ 13C TOC, biomarker indices (P alg and P aq ), published palaeoecological data and a δ 18O carbonate record from the same palaeolake. Green microalgae, freshwater macroalgae (Chara) and aquatic bryophytes were important sources of sedimentary cellulose during different phases in the environmental history of the lake. Although a strong palaeclimatic imprint can be detected in the δ 18O cellulose record from Antu Sinijärv, notably the Preboreal oscillation, the 8.2ka event and an unnamed cold oscillation ~3.25ka BP, the isotopic signal of these events may have been amplified by increases in 18O-depleted spring snowmelt. In contrast, δ 13C cellulose was tightly coupled to the Holocene evolution of terrestrial ecosystems and soils by significant inputs of biogenic carbon from the catchment and sublacustrine springs. During the early Holocene, ~11 – 9ka BP, the δ 18O cellulose and δ 18O carbonate records diverge markedly, which can be attributed to "no-analogue" seasonal, climatic, hydrological and isotopic conditions resulting from orbital forcing and residual ice-sheet impacts.

© 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction and previous work

1.1. Introduction

Here, we present stable-isotope records from the organic fraction of lacustrine marls from Lake Antu Sinijärv, Estonia, in order to investigate regional climatic and lake-basin evolution through the course of the Holocene. Use of both cellulose δ 18O and δ 13C in lake-sediment profiles is reviewed below. By employing a multi-proxy approach including geochemical analyses (C/N ratios and biomarker indices) and various published palaeoecological data-sets, we are able to refine the interpretation of the cellulose record both in terms of climatic and local (autogenic and allogenic) influences on lake biogeochemistry. In addition to calculating the oxygen-isotope values of the lake waters through the Holocene, we present a preliminary palaeotemperature reconstruction based on the relationship between mean monthly temperatures and weighted-average δ 18O data for meteoric waters at the nearest Global Network of Isotopes in Precipitation (GNIP) station. This

https://doi.org/10.1016/j.quascirev.2018.05.010
0277-3791 © 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
reconstruction relies on the uniformitarian assumptions that: 1) Antu Sinjav was always a well-mixed, open, groundwater-fed lake with a short residence time and minimal evaporation; and 2) present-day isotopic relationships are applicable throughout the Holocene. Our $\delta^{18}O$water and palaeotemperature estimates are then used to simulate the expected $\delta^{18}O$ signal in endogenic carbonate from this lake, which is compared with measured $\delta^{18}O$carbonate data from a nearby core. The contrasting isotopic records from these two materials raise important questions about the stability of isotopic relationships on millennial to multi-millennial time scales.

1.2. Isotopic analyses of lacustrine cellulose

Lake-sediment cellulose has been used as a palaeoenvironmental indicator in a diverse range of lakes of varying size and climatic setting (Wolfe et al., 2007 and references therein). They include the Great Lakes of both North America (Duthe et al., 1996; Wolfe and Edwards, 1998; Wolfe et al., 2000a) and East Africa (Beuning et al., 1997; 2002). Smaller water bodies in temperate North America, Europe, New Zealand and Patagonia have also been investigated (Edwards and McAndrews, 1989; Padden, 1996; Padden et al., 1996; Wolfe et al., 2000b; Wisse et al., 2008; Mayr et al., 2009, 2007; Lücke et al., 2009; Heikilä et al., 2010; Rozanski et al., 2010; Buhay et al., 2012; Heyog et al., 2014), as well as lakes in the circumpolar arctic/subarctic of Canada (MacDonald et al., 1993; Edwards et al., 1996; Wolfe et al., 1996, 2012; Sauer et al., 2001), Russia (Wolfe and Edwards, 1997; Wolfe et al., 1999; 2000c) and Alaska (Anderson et al., 2001), high-altitude lakes in the subtropical Andes (Abbott et al., 2000; Wolfe et al., 2001a); and the sediments of Glacial Lake Agassiz (Buhay and Betcher, 1998). However, only a few studies so far have compared the sedimentary stable-isotope records of cellulose and freshwater carbonates from hardwater lakes, in which so far these materials are co-produced (Heikilä et al., 2010; Rozanski et al., 2010; Buhay et al., 2012).

Palaeoclimatological and palaeohydrological applications of the $\delta^{18}O$ values of lake-sediment cellulose ($\delta^{18}O_{\text{cellulose}}$) developed to circumvent the problems inherent in attempting to obtain meaningful $\delta^{18}O$ time series from bulk organic matter (Meyers and Teranes, 2001). Interpretation of the latter is severely complicated by the contribution of oxygen from the oxygen-bearing compounds used to oxidise organic matter to CO$_2$ prior to isotopic analysis (Meyers and Teranes, 2001; Meyers and Lallicher-Verges, 1999). Oxygen- and carbon-isotope analyses of cellulose have proven particularly useful in carbonate-free, soft-water lakes (Wolfe et al., 2001b).

Oxygen-isotope analyses of aquatic cellulose have been used to investigate past variations in the water balance of lakes and their relationship to past changes in climate by documenting shifts along the meteoric water line (MWL), which expresses the correlation between $\delta^{18}O$ and $\delta$ in precipitation (Craig, 1961; Dansgaard, 1964; Rozanski et al., 1993), or along a local evaporation line (LEL) in lakes where evaporation is important (Craig and Gordon, 1965; Confantini, 1985). This displacement from the MWL in $\delta^{18}O$/$\delta$D space occurs because evaporation affects the $^{18}O$/$^{16}O$ and $D$/$H$ systems differently (Dansgaard, 1964; Confantini, 1985). Stable carbon isotopes in lacustrine cellulose have also been used to elucidate the relationship between past hydrological changes and lake/catchment carbon cycling (Wolfe et al., 2001a,b).

Coupled analyses of the oxygen- and carbon-isotope composition of lake-sediment cellulose were first undertaken by Edwards and McAndrews (1989); who studied the Holocene palaeohydrology of a Canadian Shield lake in southern Ontario. They argued that lake-sediment $\delta^{18}O_{\text{cellulose}}$ could be used as a tracer for lake-water $\delta^{18}O$, through the application of the constant (species- and temperature-independent) cellulose-water fractionation factor, $\alpha$, that had previously been demonstrated between terrestrial cellulose and water (e.g. Edwards et al., 1985). This fractionation factor has also been shown to apply to submersed aquatic plants irrespective of assumed metabolic pathway (DeNiro and Epstein, 1981; Sternberg et al., 1984). The Edwards and McAndrews (1989) study, combined with previous isotopic investigations in southern Ontario comparing terrestrial cellulose and lacustrine carbonates (Edwards et al., 1985; 1986; Edwards and Fritz, 1986; 1988) led to the conceptual recognition of analogous oxygen-isotope relationships between terrestrial cellulose, lacustrine endogenic carbonate, aquatic cellulose and their respective source waters (Wolfe et al., 2001a,b). The following equations summarise these relations for terrestrial-plant cellulose (Eq. (1)), endogenic carbonate (Eq. (2)) and aquatic cellulose (Eq. (3)):

$$\delta^{18}O_{\text{cellulose}} = \delta^{18}O_{\text{mw}} + \varepsilon^{18} \text{evap} + \varepsilon^{18} \text{cellulose-leafwater}$$  

(1)$$
\delta^{18}O_{\text{carbonate}} = \delta^{18}O_{\text{mw}} + \varepsilon^{18} \text{hydro} + \varepsilon^{18} \text{carbonate-lakewater}$$  

(2)$$
\delta^{18}O_{\text{cellulose}} = \delta^{18}O_{\text{mw}} + \varepsilon^{18} \text{hydro} + \varepsilon^{18} \text{cellulose-lakewater}$$  

(3)$$
$$
where $\delta^{18}O_{\text{mw}}$ is the isotopic composition of local meteoric water, $\varepsilon^{18} \text{evap}$ is the isotopic enrichment due to kinetic and equilibrium effects during transpiration in terrestrial plant leaves, $\varepsilon^{18} \text{hydro}$ is the isotopic enrichment in lakes due to hydrological factors (notably evaporative enrichment), $\varepsilon^{18} \text{cellulose-leafwater}$ and $\varepsilon^{18} \text{cellulose-lakewater}$ are the respective, temperature-independent biochemical enrichments that occur during cellulose synthesis, and $\varepsilon^{18} \text{carbonate-lakewater}$ is the temperature-dependent enrichment between lake water and endogenic carbonate (Wolfe et al., 2001a,b; 2007).

Edwards and McAndrews (1989) also argued that the fine-grained cellulose fraction of lake sediments was primarily of aquatic origin, based on: a) the coherent isotopic relations between surface-sediment cellulose and modern lake water; b) overlapping $\delta^{18}O$ time series from two widely separated sediment cores; and c) the lack of overlap between the $\delta^{13}C$ time series from the same cores, which is not surprising given the much broader range of $\delta^{13}C$ values encountered in aquatic plants and algae compared with terrestrial C$_3$ plants (Rau, 1978; Sharkey and Berry, 1985; Keeley and Sandquist, 1992); as well as d) a consideration of the expected $\delta^{18}O$ values for terrestrial and aquatic plants, based on measurements of modern terrestrial-plant cellulose from the study area. Their mass-balance calculations led to the conclusion that aquatic sources contributed ~90% of the fine-grained sediment cellulose under modern conditions.

In summary, two key conclusions were reached by the Edwards and McAndrews (1989) study: 1) that the biochemical fractionation between aquatic cellulose and source water was constant and well understood; and 2) that the fine-grained (<150 µm) cellulose fraction of lake sediments was predominantly derived from aquatic plants and/or algae. These conclusions have subsequently become fundamental assumptions in the routine estimation of $\delta^{18}O_{\text{cellulose}}$ from $\delta^{18}O_{\text{cellulose}}$. Many subsequent studies that utilised this method simply referred back to Edwards and McAndrews (1989) (MacDonald et al., 1993; Wolfe et al., 1996; Duthe et al., 1996; Wolfe and Edwards 1997; Buhay and Betcher, 1998). However, later work drew more heavily on C/N ratios and $\delta^{13}C$ values of cellulose and/or total organic carbon ($\delta^{13}C_{\text{TOC}}$) in order to demonstrate a largely aquatic source for the sedimentary-cellulose fraction (Wolfe et al., 1999; 2000c; 2001a,b; Abbott et al., 2000; Anderson et al., 2001). Comparisons between $\delta^{18}O_{\text{cellulose}}$ values of surface sediments, modern submersed aquatic-plant material and $\delta^{18}O$ values of modern lake water have also commonly been used as a “calibration” method in order to support (or constrain) both of these assumptions. However, the influence of seasonal hydrological
processes needs to be considered in surface-sediment calibrations in the case of studies that rely on a single measure of lake-water isotope composition (Wolfe and Edwards, 1997; Wolfe et al., 2001a).

1.3. Sources of cellulose in lake sediments

Given the diversity of potential aquatic- and terrestrial-cellulose sources, it is essential to consider the full range of possibilities in the light of the available indicators of sediment composition in any individual lake, such as C/N ratios, $\delta^{13}$C values, biomarker indices and palaeoecological data.

1.3.1. Algae

Despite the assertions by Meyers and Ishiwatari (1993a; 1993b) and Meyers (1994; 1997) that algae do not contain cellulose, they often represent an important source of cellulose for lake sediments (Wolfe et al., 2001a,b). Emphasis is usually placed on the green microalgae (Chlorophyta), including the Zygnematales and Desmidiales, and the freshwater green macroalgae or stoneworts (Charophyta) (e.g. Wolfe et al., 2001a,b).

In the green algae, cellulose is usually the main structural polysaccharide (Lee, 1989). In some taxa, however, it may be replaced by xylan (Frei and Preston, 1964; Dodge, 1973), mannan (Frei and Preston, 1961; Mackie and Preston, 1968) or glucan (Lee, 1989). A full review of the numerous taxa that contain cellulose is beyond the scope of this discussion. However, they include such common microalgal genera as Pediasstrum, Cladophora, Chara, Botryococcus, Scenedesmus and Oocystis, and the freshwater macroalgae Chara and Nitella (Prescott, 1969; Dodge, 1973; Tsekos, 1999).

Other algal taxa commonly or occasionally found in lakes that contain some cellulose-producing species include the Chrysophyceae (golden algae), which are important in soft waters, Xanthophyceae (yellow-green algae), Cryptophyta (cryptomonads), Haptophyta (haptophytes), Dinoflagellata (dinoflagellates), Rho-dophyta (red algae) and Phaeophyceae (brown algae) (Brown et al., 1969; Brown et al., 1970; Dodge and Crawford, 1970; Chapman and Chapman, 1973; Dodge, 1973; Sheath et al. 1975; Trainor, 1978; Herbdt and Zugenmaier, 1979; Kumar and Singh, 1979; Lee, 1989; Zeeb and Smol, 2001).

1.3.2. Submerged and floating aquatic macrophytes

Among the macroscopic contributors to the sedimentary cellulose pool, submerged aquatic macrophytes often dominate in shallow lakes and littoral sediments. These include vascular plants such as Myriophyllum, Ceratophyllum, Potamogeton, Callitriche and Littorella, and aquatic mosses such as Fontinalis, Drepanocladius and Scorpidium. Macrophytes with floating leaves (notably the water-lilies Nymphaea and Nuphar) are often significant cellulose sources. Taxa with floating waxy leaves (e.g. Nymphaea) may be considered “honorary” submerged macrophytes with respect to their characteristic mid-chain-length lipid biomarkers (Ficken et al., 2000). Submerged and floating aquatic plants commonly contribute cellulose to profundal sediments through resuspension of littoral deposits during lake-level lowstands.

1.3.3. Emergent macrophytes and terrestrial vegetation

Emergent aquatic vegetation potentially contributes large amounts of cellulose to sediments in smaller lakes and/or marginal environments. Members of the Poaceae (seeds, e.g. Phragmites), Cyperaceae (sedges, e.g. Carex, Cladium) and Typhaceae (Typha) are usually the most important taxa. Allochthonous terrestrial plant material (seeds, leaves, bud scales, twigs etc.) derived from the lake’s watershed is another potentially large contributor of cellulose to lake sediments. This source may be particularly significant in lakes surrounded by forest, or fed by large streams or rivers.

An underestimated (terrestrial) source of cellulose is pollen (and possibly cryptogam spores). While the outer layer (exine) of the walls of pollen grains is composed of sporopollenin, the inner layer (intine) consists of cellulose (Shaw and Yeaden, 1964; Moore et al., 1991; Loader and Hemming, 2004). While intine may only be a minor component of the cellulose pool, its contribution should certainly be considered in lakes with low aquatic productivity, a slow sedimentation rate and/or a high pollen influx. Moreover, cellulose from this source may be preferentially preserved within the highly resistant exine in sediments where diagenesis impairs the preservation of aquatic cellulose. Similarly, bacterial sources of cellulose (e.g. Acetobacter) from soils or from within lakes do not appear to have been adequately explored.

1.4. Cellulose-water oxygen-isotope fractionation

Many laboratory and field studies have attempted to quantify the relationship between the $^{18}$O/$^{16}$O ratios of aquatic cellulose and those of the water in which the organisms grew (Epstein et al., 1977; DeNiro and Epstein, 1981; Sternberg and DeNiro, 1983a; Sternberg et al., 1984; 1986; Yakir and DeNiro, 1990; Abbott et al., 2000). The majority suggest that fractionation between water and cellulose is constant and independent of temperature (DeNiro and Epstein, 1981), plant species, photosynthetic pathway (Epstein et al., 1977; Sternberg and DeNiro, 1983a; Sternberg et al., 1984; 1986), growth conditions (Yakir and DeNiro, 1990) and the $\delta^{13}$C value of dissolved CO$_2$ (due to its equilibration with water in the cell prior to fixation) (DeNiro and Epstein, 1979).

A constant $\delta^{18}$O fractionation factor, $\alpha$, has been observed between water and cellulose in a variety of aquatic organisms, including freshwater algae and vascular plants (e.g. Epstein et al., 1977; DeNiro and Epstein, 1981). A value of $\alpha = 1.028 \pm 0.003$ was observed in the majority of these studies. This results in a consistent $^{18}$O enrichment in aquatic cellulose with respect to its source water, $\varepsilon^{18}$cellulose-lakewater = 27–28‰ (Yakir, 1992; Sternberg, 1989), which has been attributed to the isotopic signature of the water being “fixed” during the carbonyl hydration reaction (e.g. Sternberg and DeNiro, 1983b). The majority of these studies, however, have focused on temperate environments. Work in tropical East Africa has suggested a smaller fractionation factor, $\alpha = 1.025 \pm 0.003$ (Reuning et al., 1997; 2002). Their results imply a small temperature effect on the fractionation factor; more work is needed to clarify this possibility (Wolfe et al., 2001a,b, Waterhouse et al., 2013).

Comparisons between surface-sediment samples, overlying lake water and modern aquatic vegetation are often used in cellulose $\delta^{18}$O studies in order to validate the technique (as well as the cellulose source). In open lakes with perennial outflows, single-episode surface-sediment calibration may produce coherent results (e.g. Abbott et al., 2000), but where strong seasonal variations in the isotopic composition of lake waters occur (e.g. where snowmelt is important) the contrasting temporal resolution of the sediment (a weighted average for the whole year) and water data (instantaneous) may result in a significant offset between results from the two data sources (Wolfe and Edwards, 1997; Wolfe et al., 2001a,b). Isotopic monitoring of the lake water/vegetation throughout the entire growing season is therefore desirable but was not feasible in this study.

1.5. Lipid biomarkers

Although C/N ratios are widely employed to distinguish between organic matter of terrestrial and aquatic origin (Meyers and Ishiwatari 1993a,b; Meyers and Lallier-Vergès, 1999), C/N on its
own is a blunt instrument due to the diversity of possible sources contributing to lake sediments and the impacts of early diagenesis (Tyson, 1995). In this paper, we also deploy two groups of lipid biomarkers to clarify the origin of the sedimentary cellulose. The organic fraction of lacustrine sediments comprises a mixture of autochthonous compounds synthesized by living organisms that lived within the lake, such as algae, bacteria and submerged/ floating macrophytes, with allochthonous inputs derived from the catchment, notably from vascular land plants. n-Alkanes, which are least susceptible to degradation, are most commonly used in palaeoenvironmental reconstructions (Meyers, 1997; Meyers and Ishiwatari, 1993a,b). Suites of n-alkanes characteristic of certain plant groups can be used to investigate the relative importance of terrestrial versus aquatic sources. Odd-numbered, long-chain homologues (C27–C35) are generally characteristic of terrestrial higher-plants leaf waxes (Eglinton and Hamilton, 1967), whereas short-chain homologues (C17–C21) are characteristic of aquatic algae (Cranwell et al., 1987), but vulnerable to diagenesis. Ficken et al. (2000) demonstrated that odd-numbered, mid-chain-length (C23, C25) n-alkanes formed the dominant component of leaf waxes produced by submerged and floating aquatic plants (which include the macroalga Chara) according to Nunez et al. (2002) and Mead et al. (2005), although Ortiz et al. (2015) found that it maximized at C18. Hence, Ficken et al. (2000) proposed the n-alkane Pm proxy (Pm = (C23 + C25)/(C23 + C25 + C28 + C29)), with values that theoretically range from 0 to 1, to distinguish the relative contribution of leaf waxes derived from submerged/floating aquatic macrophytes from long-chain compounds produced by emergent aquatic/ terrestrial plants. A Pm value >0.4 signifies that a significant proportion of the n-alkane fraction originated from submerged/floating plants (Ficken et al., 2000).

Zhang et al. (2004) formulated a similar proxy for algal inputs into lake sediments, based on the proportion of algal n-alkanes plus a hydrocarbon compound produced by Botryococcus braunii (cyclobotryococcatriene), relative to terrestrial-plant leaf waxes: Palg = [(C23:1 + C25:1 + C27:1 + cyclobotryococcatriene)/(C23:1 + C25:1 + C27:1 + cyclobotryococcatriene) + C29 + C31 + C33]). The Palg formula was revised by Cockerton et al. (2015) for application to the sediments of Lakes Victoria and Edward, East Africa, which did not contain cyclobotryococcatriene: Palg = (C23:1 + C25:1 + C27:1)/(C23:1 + C25:1 + C27:1 + C29 + C31 + C33). Here, we apply a version of the Palg proxy applicable to the more abundant, even-numbered, n-alkanoic acids found in the Antu Sinijärv sediment extracts: Palg = (C16+C18)/(C16+C18+C26+C28) (cf. Meyers, 1997). Assuming good preservation of the easily degraded, short-chain homologues (Napolitano, 1999; Ortiz et al., 2015), higher values of this index would imply a predominantly algal source for the sedimentary organic matter, with the reservation that Chara may produce significant amounts of short-chain (C16 and C16:1) fatty acids (Nunez et al., 2002). However, it is possible to say with more confidence that low values of both Pm and Palg suggest a substantial contribution of leaf waxes derived from terrestrial plants and/or emergent macrophytes.

2. Site description, materials and methods

2.1. Site description

2.1.1. Late-glacial and Holocene history of the region

Lake Antu Sinijärv (Antu Blue Lake) (59.3.8’N; 26.14.5’E; 94.6 m a.s.l., 2.4ha) is a shallow, calcareous lake (maximum depth 7.3 m), forming one of the Antu group of lakes located on the southern slopes of the Pandivere Uplands in Eastern Estonia (Fig. 1). This area was ice-covered during the last glacial, but became largely ice-free during the Allerød interstadial, with the final disappearance of local ice and resulting meltwater by ~13.3ka at the latest (all dates quoted in this paper are in calendar years Before Present, unless otherwise stated) (Amon et al., 2016). However, the Fennoscandian Ice Sheet, which disappeared ~10.5ka (Cuzzone et al., 2016), may have continued to influence the climate of the Uplands even after glacial ice receded from Estonia. In addition, the Baltic Sea and its precursors had the potential to impact regional climate: these waterbodies varied in size and salinity as a result of ice-sheet melting, local and regional isostatic changes, and variations in global ocean volume (Berglund et al., 2005). Following deglaciation and prior to the start of Holocene warming, the Baltic Ice Lake

Fig. 1. Right. Map of Estonia and surroundings, showing the location of the Antu group of lakes and other sites referred to in the text. Left. Lakes Antu Sinijärv, Rohejärv and Valgejärv, showing the locations of the ANTU1 (black triangle) and ANTU3 (black square) core sites and the former extent of Palaeolake Antu (heavy line) (Lehtmaa, 2006), with contours in metres.
occupied the basin. This was replaced by the Yoldia Sea, an initially brackish water body that persisted until ~10.7ka. The early, brackish phase of the Yoldia Sea corresponded to a brief cold reversal known as the Preboreal oscillation (PBO), which began ~11.3ka and lasted for 150–250yr (Björck et al., 1996; Fisher et al., 2002). Between 10.7 and 9.8ka, the cold, freshwater Ancylus Lake occupied the Baltic basin, to be replaced by the Littorina Sea, a variably brackish waterbody that has endured to the present day (Berglund et al., 2005).

The temperature of the Baltic region rose sharply after the PBO in response to orbital forcing and ice-sheet recession; winter and summer temperatures rose by ≤ 12 °C and ≤ 5 °C, respectively, until ~8ka according to Veski et al. (2015), with a proposed Holocene Thermal Maximum (HTM) lasting from ~8 to ~4.5ka (Seppä and Poska, 2004), although some authors have argued for a somewhat earlier onset (Valiranta et al., 2015). Early to mid-Holocene warming was interrupted by a short-lived cooling, assumed to correspond to the 8.2ka event, during which temperatures in Eastern Estonia fell by ≤ 2 °C for ~200 years, in association with increased anticyclonic conditions and a weakening of zonal airflow (Veski et al., 2004; Heikkilä and Seppä, 2003). Progressive cooling characterized the interval after the HTM, from ~4.5ka onwards (Seppä and Poska, 2004).

Low lake levels at the start of the Holocene in the eastern Baltic region suggest dry conditions (Saarse and Harrison, 1992); in contrast, rising lake levels through the HTM point to wetter conditions, although Seppä and Poska (2004) have suggested, based on palynological evidence, that the HTM in Estonia was characterized by warmer, drier summers as a result of increased anticyclonic conditions accompanied by enhanced meridional airflow. Reconstructed water-level changes for Antu Sinijärv (Sohar and Kalm, 2008) appear out of phase with those from the eastern Baltic during the early Holocene (Saarse and Harrison, 1992), possibly as a result of dating issues with earlier cores from the lake, although the reconstruction of a low-water phase during the HTM agrees with Seppä and Poska (2004).

Changes in temperature and effective moisture have led to marked shifts in vegetation on the Pandivere Uplands. Human impact became increasingly important from the mid- to late Holocene onwards. Treeless tundra vegetation during the cold, Younger Dryas stadial (YD) prior to ~11.5ka was replaced by dwarf Betula woodland during the earliest Holocene until ~10ka, when Pinus began to dominate, with other broadleaved taxa appearing by ~9.5ka (Punning et al., 2000; Aman et al., 2016). Deforestation, as a result of human impact, began in the mid-Holocene ~5.7ka (Saarse and Liiva, 1995) and occurred in several phases, although Antu Sinijärv is still surrounded by mixed stands of Scots Pine (Pinus sylvestris) and Norway Spruce (Picea abies).

2.1.2. Modern limnology and climatology

Today, Lake Antu Sinijärv is fed by direct precipitation and by groundwater from an Ordovician limestone aquifer that discharges into the lake through at least seven submerged spring vents, which are ≤2 m deep (Saarse and Liiva, 1995). It drains southwards through Lakes Rohejärv (also known as Vahejärv) and Väljäjarv, with which it was united before ~4ka (Nedelskaja, 2011) to form a single open lake, Palearloake Antu, which covered ~14.4ha. Palearloake marls extend up to ~2 m above the present lake (Lehtmaa, 2006) (Fig. 1). Today, Antu Sinijärv has an estimated water-residence time of ~2–3 months (Saarse and Liiva, 1995).

The climate of the region is characterized by warm/wet summers and cold/wet winters, with cool/drier conditions in spring and autumn. Annual-mean air temperature (MAT) for the period 1981–2010 at Väike-Maarja meteorological station, ~7 km north of the lake (Fig. 1) was 4.9 °C, with minimum and maximum monthly values, respectively, of ~4.5 °C in February and 17.4 °C in July. Annual total precipitation was 693 mm over the same period, with maxima in August and October and a minimum in April. Relative humidity is high all year, peaking at 88% in November through to January (Estonian Weather Service: http://www.ilmateenistus.ee/).

The water temperature of Lake Antu Sinijärv ranges from 6.4 to 18.0 °C. A thermocline is typically found at a depth of 1–2 m (Maemets, 1977). The lake has a pH of 7.4–8.0 and an HCO3 content of 262–274 mg L−1 (Laumets et al., 2014). It is surrounded by a narrow strip of peat on all but the eastern shore (Saarse and Liiva, 1995). It supports dense stands of submerged aquatic macrophytes, including the green macroalga Chara, the brown moss Scorpidium, and the vascular plants Myriophyllum verticillatum and Ceratophyllum demersum (Saarse and Liiva, 1995; Olsson and Kaup, 2001).

2.1.3. Sediment record

The lacustrine deposits, which vary from 5.1 m thick in the southern part of the basin to 3.3 m thick in the northern basin, consist of marl, calcareous mud and gyttja. Previous studies have investigated pollen (Saarse and Liiva, 1995; Punning et al., 2000; Nedelskaja, 2011; Laumets et al., 2014), plant macrofossils and non-pollen palynomorphs (Nedelskaja, 2011; Laumets et al., 2014), carbonate isotope values (Punning et al., 2000; Nedelskaja, 2011; Laumets et al., 2014) and ostracods (Sohar and Kalm, 2008), based on several cores recovered from the lake. No previous stable-isotope studies of the organic fraction have been conducted. Unfortunately, the age models for previous cores (apart from Nedelskaja, 2011) were based almost entirely on 14C dates from aquatic material, which suffers from significant reservoir effects in freshwater lakes (Olsson and Kaup, 2001; Sensula et al., 2006). Our investigation aimed to provide new insights into local and regional environmental histories using a more reliable chronology based exclusively on terrestrial-plant macrofossils (see Supplementary Data).

2.2. Methods

Here, we provide a short overview of the field and laboratory methods used: full details are provided in Supplementary Data. Investigations of oxygen and hydrogen isotopes in modern rainwater, groundwater and lake water were undertaken in order to aid the interpretation of isotope data from the lake sediments. Modern precipitation and groundwater samples were collected at Väike-Maarja and lake-water samples close to the lake centre (Fig. 1). The entire Holocene lake-sediment record (core ANTU1-2) was recovered beneath 4.1 m of water using a Russian corer and an age model developed using radiocarbon ages; only terrestrial macrofossils were used in order to avoid water errors. The core sediments were sampled at typical intervals of 1–4 cm and analysed for bulk sediment composition; total organic carbon (TOC), total nitrogen (TN) and C/N ratio; and cellulose isotope values. In a novel step, PAn and Pao were analysed on the solvent extracts remaining after cellulose preparation. As a first approximation, Holocene variations in air temperature were estimated from δ18Owater and the modern relationship between weighted-average δ18Owater and mean air temperature (MAT) (cf. Edwards et al., 1996; von Grafenstein et al., 1996) although we recognize that this relationship may be complicated by seasonal covariance between temperature and precipitation amount (Tyler et al., 2016). Since core ANTU1-2 contained refractory organic matter which made it hard to obtain a clean carbonate fraction for isotope analysis, its δ18Ocellulose record is compared below with the δ13C carbonate curve for core ANTU3 (Nedelskaja, 2011; unpubl. data), the only other carbonate record from Palearloake Antu with a chronology based entirely on
terrestrial macrofossils. The location of ANTU3 is shown on Fig. 1.

3. Results and interpretation

3.1. Stable isotopes in precipitation, lake water and groundwater

Monthly precipitation $\delta^{18}O$ ($\delta^{18}O_{\text{nw}}$) values for Väike-Maarja vary from $-14.5\%$ (December) to $-9.8\%$ (June), although data are lacking for January to April, inclusive (Fig. 2a). However, $\delta^{18}O_{\text{nw}}$ values from the most representative GNIP station at Tartu, $-100$ km to the south (Fig. 1), provide a more complete record. Here, $\delta^{18}O_{\text{nw}}$ values range from $-14\%$ (January) to $-7.9\%$ (June); for the months for which data are available for both sites, there is reasonable agreement (albeit values are not for the same years) (Fig. 2a). For comparison, Figure 2a also shows the data for Vilsandi, a coastal GNIP station. At Tartu, there is a close relationship between monthly MAT and $\delta^{18}O_{\text{nw}}$ values ($T = 2.1 \times \delta^{18}O + 30.1$; $r^2 = 0.54$. See Supplementary Data). The precipitation data from Tartu fall on a local meteoric water line (LMWL) described by $\delta D_{\text{nw}} = 8.01 \times \delta^{18}O_{\text{nw}} + 8.57$, and have weighted mean-annual values of $-10.51\%$ for $\delta^{18}O_{\text{nw}}$ and $-75.45\%$ for $\delta D_{\text{nw}}$, respectively. Väike-Maarja rainfall values fall close to the LMWL for Tartu (Fig. 2c). Deuteron-excess ($\delta D_{\text{nw}}$) values for Väike-Maarja precipitation vary between about $+7\%$ in June and about $+12\%$ in December and May; the more complete record for Tartu shows low values (around $-4\%$) in March, with higher values in August–December, peaking around $+11\%$, suggesting some autumal recycling of surface water to the atmosphere (Fig. 2b).

Today, Antu Sinijärv lake waters barely diverge from the LMWL for Tartu (Fig. 2c), implying limited isotopic evolution through evaporation, in contrast to many other modern Estonian lakes reported by Stansell et al. (2017), although this behaviour may not have applied throughout the Holocene. Antu Sinijärv also exhibits damped seasonal variations in $\delta^{18}O$, with lake-water values ranging from $-11.5$ to $-12.2\%$ (Fig. 2d). However, two samples of snow and snowmelt collected from the surface of the lake ice in February 2005 yielded $\delta^{18}O$ values of $-23.98$ and $-14.15\%$, respectively. Slight isotopic depth stratification probably reflects both minor surface evaporative enrichment during summer/autumn and groundwater inflow from the submerged springs (Fig. 2d). Groundwater $\delta^{18}O$ values also plot very close to the LMWL. They averaged $-12 \pm 0.16\%$.

3.2. Lake sediments

Overlapping cores ANTU1 and ANTU2 were aligned stratigraphically (see Supplementary Data) in order to provide a composite sequence (ANTU1+2), together with the short surface core SURF, for plotting and data description (Figs. 3, 4). All plots presented here are based on corrected depths following stratigraphical alignment. The major sedimentary units and the corresponding environmental conditions reconstructed from published studies are described below.

Dark lacustrine silt (450 – 428 cm; 11.4 – 10.8ka BP). The basal unit is a minerogenic lake mud (residue $\leq 90\%$), with very low CaCO$_3$ (3–7%) and TOC contents (3–6%). Low C/N ratios (11–13), moderately high P$_{\text{ag}}$ (0.35–0.77) and low P$_{\text{aq}}$ values (0.28–0.41) suggest a mixture of lacustrine (mainly microalgal) and minor terrestrial cellulose sources. Low pollen concentrations (Betula incl. nana, Pinus, Salix and possibly reworked grains of Picea and temperate trees), with some charcoal (Laumets et al., 2014), suggest a cold, open landscape. $\delta^{13}C$ values for both TOC and cellulose (–30 to $-25\%$ and $-23$ to $-20\%$, respectively) (Fig. 5a), are normal for lacustrine organic matter derived from atmospheric CO$_2$, $\delta^{13}C_{\text{cellulose}}$ is low ($6$–$13\%$), corresponding to reconstructed $\delta^{18}O_{\text{lakewater}}$ values ($-21$ to $-15\%$) and cellulose-inferred palaeotemperatures ($-14$ to $-1^\circ$C) much lower than the present day (Fig. 6).

Cytjä (427 – 413 cm; 10.8 – 10.4ka BP). This distinctive organic lake mud (cf. Saarse and Liiva, 1995, Sohar and Kalm, 2008, and Laumets et al., 2014), still has a low CaCO$_3$ content (3–6%) but a much higher TOC content (30–41%) (Figs. 3, 4). C/N ratios (12–13), P$_{\text{ag}}$ (0.32–0.66) and P$_{\text{aq}}$ (0.40–0.70) suggest dominantly aquatic organic-matter sources with minimal inputs from terrestrial ecosystems. This inference is confirmed by the presence of green, colonial, planktonic microalgae (Botryococcus), benthic macroalgae (Characeae) and pollen of vascular aquatic macrophytes (the waterlilies Nuphar and Nymphaea, and the emergent Typha: Nedelskaja, 2011; Laumets et al., 2014). Although Botryococcus has a wide tolerance (Tyson, 1995), it is common in European sediments of late-glacial to Preboreal age, where it reflects cold, oligotrophic-dystrophic conditions (Jankovská and Komárek, 2000). $\delta^{13}C_{\text{TOC}}$ (–36 to $-30\%$) and $\delta^{13}C_{\text{cellulose}}$ values (–33 to $-26\%$) are transitional between those found in the basal mud and the overlying marls (Fig. 5c). $\delta^{13}C_{\text{cellulose}}$ (17–19%) and $\delta^{18}O_{\text{lakewater}}$ (–11 to $-9\%$) both reach values higher than today, suggesting annual MATs (7–11 C) similar to, or higher than today’s (Fig. 6). In apparent conflict, the surrounding vegetation reported by Laumets et al. (2014) was an open Betula–Pinus forest with a high proportion of non-arboreal pollen (notably Artemisia and Chenopodiaceae, with some Poaceae and Cyperaceae), together with pollen and macrofossils of subarctic shrubs and herbs (Betula nana, Salix, Dryas octopetala), and abundant microscopic charcoal (Laumets et al., 2014). A highly seasonal climate with cold winters and warm, dry summers can be inferred. This was associated with near-maximal orbital forcing and probably also increased winter ice cover over the freshwater Ancylus Lake to the north (Punning et al., 2000).

Pale marl (412 – 365 cm; 10.4 – 9.1ka BP). This unit marks the onset of Holocene marl deposition at the ANTU1+2 core site (Fig. 5d). The CaCO$_3$ content rises to 38–91%, with a variable organic content (TOC 37–42%). The origin of the organic matter was dominantly aquatic with a high algal contribution (C/N ratio 11–13, P$_{\text{ag}}$ 0.64–0.89, P$_{\text{aq}}$ 0.55–0.68), represented by the colonial green microalgae Coelastra rum reticulatum, Pedias trum boreanum and Botryococcus, together with a modest presence of Chara oogonia and Nuphar pollen (Nedelskaja, 2011; Laumets et al., 2014). The association of Coelastrum and Pedias trum, with Metacypris cordata dominating the ostracoda fauna, implies warmer but relatively shallow, mesotrophic-eutrophic conditions in the lake (Happey- Wood, 1988; Jankovská and Komárek, 2000; Sohar and Kalm, 2008), borne out by a rise in TN content to 2.9–3.7%. Betula–Pinus forest initially surrounded the site, with an increasing presence of temperate deciduous trees: Ulmus, Corylus and Alnus (Nedelskaja, 2011; Laumets et al., 2014). $\delta^{13}C_{\text{TOC}}$ and $\delta^{13}C_{\text{cellulose}}$ decreased to unusually low values (–41 to $-37\%$ and $-39$ to $-33\%$, respectively: see Discussion). High $\delta^{18}O_{\text{cellulose}}$ values (16–17.5%) suggest $\delta^{18}O_{\text{lakewater}}$ (–12 to $-10\%$) and annual MATs (6–9 C) similar to the present or a little higher (Fig. 6).

Dark organic marl (364 – 328 cm; 9.1 – 7.6ka BP). This unit exhibits consistently high CaCO$_3$ (48–87%) and TOC contents (17–47%). Slightly increased C/N ratios (10–15), coupled with declines in both P$_{\text{ag}}$ (0.43–0.77) and P$_{\text{aq}}$ (0.39–0.72), imply a modest increase in the input of terrestrial detritus. Aquatic cellulose sources are represented by low counts of Botryococcus, accompanied by Nuphar pollen and abundant Chara oogonia (Nedelskaja, 2011; Laumets et al., 2014). At the base, the pollen assemblage is similar to the underlying unit, but with an increasing representation of Alnus, Corylus and thermophilous broad-leaved trees (Ulmus, Tilia, Fraxinus). A short-lived dip in Alnus, Corylus and Ulmus pollen, accompanied by a small peak in Upland Herbs (Nedelskaja, 2011),
may mark the 8.2ka BP cold event. $\delta^{13}C$ values continued to be unusually low ($-43$ to $-38\%$ for both TOC and cellulose). In contrast, $\delta^{18}O_{\text{cellulose}}$ exhibited a marked perturbation in the middle of this unit which we attribute to the 8.2ka BP event (Figs. 3,4; see Discussion). Estimated MATs varied widely from $-4$ to $+12 \, ^\circ\text{C}$ (Fig. 6).

**Grey marl (327 – 294 cm; 7.6 – 6.6ka BP).** The grey marl displays similar bulk characteristics to the underlying dark organic marl, but with uniformly high CaCO$_3$ (87–92%) and TOC (37–42%) contents. Low C/N ratios (10–12), $P_{\text{alg}}$ (0.38–0.73) and $P_{\text{aq}}$ (0.26–0.52) suggest a mixture of aquatic (mainly algal) and minor terrestrial cellulose sources. *Botryococcus* and *Cosmarium* increased

---

Fig. 2. Modern water-isotope data. (a) Climate and $\delta^{18}O_{\text{w}}$ values from Global Network of Isotopes in Precipitation (GNIP) stations at Tartu and Vilsandi, and from Väike-Maarja (this study). (b) Deuterium-excess values ($d_{\text{me}}$) in precipitation from the three precipitation isotope series. (c) $\delta^{18}O - \delta^D$ biplot for lake water from Antu Sinijärve and for precipitation and groundwater from Väike-Maarja. Lake-water data with $\delta^{18}O$ values $<-14\%$ relate to snow and melted water on the frozen lake surface. Local meteoric water lines (LMWL) for Väike-Maarja ($\delta^D - 7.33 \times \delta^{18}O + 2.54$) and Tartu ($\delta^D - 8.01 \times \delta^{18}O + 8.57$) are also shown. (d) Depth profiles of $\delta^{18}O_{\text{lake water}}$ for Antu Sinijärve.
rapidly in this zone (Nedelskaja, 2011). *Cosmarium* is a large, single-celled, non-motile green microalga (desmid) that may occupy benthic niches, often in association with aquatic plants, and tends to be most common in summer to autumn (Happey-Wood, 1988). Laumets et al. (2014) found large numbers of *Chara* oogonia in their core, together with pollen of the emergent macrophytes *Juncus* and *Cladium*. The surrounding vegetation was a moist, temperate forest dominated by *Betula*, *Alnus* and thermophilous broad-leaved trees.
However, modest percentages of Pinus pollen and increasing numbers of Poaceae and Upland Herbs suggest the presence of conifers and open areas within the wider landscape (Nedelskaja, 2011). Anomalously low $\delta^{13}$C values continued (TOC: −41 to −39‰; cellulose: −41 to −38‰), $\delta^{13}$O cellulose (15−17‰) and reconstructed $\delta^{18}$O water values (−13 to −11‰) suggest MATs of 3−8°C (Fig. 6), spanning modern observations.

Pale laminated marl, Organic marl, Pale marl, Dark laminated marl (293 − 215 cm; 6.6 − 3.5ka BP). This section is characterized by abundant to very abundant charophyte oogonia. CaCO₃ contents ranged from 70 to 86%, and TOC from 30 to 43%. C/N ratios remained very steady at 10−12, within the range observed in modern Chara (unpubl. ISOMAP-UK data; Aichern et al., 2010), while $P_{al}$ and $P_{aq}$ were 0.18−0.69 and 0.33−0.53, respectively, suggesting some terrestrial input. During this interval, Botryococcus increased markedly, in association with Cosmarium; however, the latter declined after ~5ka BP. Stands of floating (Nuphar, Nymphaea) and submerged vascular macrophytes (Potamogeton) occupied the shallows (Nedelskaja, 2011). Significant changes also occurred in the wider landscape. Picea, Pinus and Quercus increased, while Betula, Alnus and thermophilous trees (Ulmus and Tilia) gradually declined (Nedelskaja, 2011). Signs of human impact also appeared after ~5ka BP, in the form of sporadic cereal grains, Calluna, Pteridium, weeds of cultivation and microscopic charcoal (Laumets et al., 2014). The appearance of Sphagnum and Equisetum spores, together with Cyperaceae and marshland herbs (Menyanthaceae), records the onset of paludification. Peak growth of Antu Sinijarvi from Vahejärvi after the level of Palaeolake Antu fell around 4ka BP (Nedelskaja, 2011). Carbon-isotope values remained persistently low, $\delta^{18}$O cellulose (14−17‰) and reconstructed $\delta^{18}$O water values (−14 to −11‰) imply little change in MATs (1−8°C), apart from a slight cooling after ~4.3ka (Fig. 6).

Palm marl, Marl with plant remains, Dark laminated marl, Pale laminated marl (214 − 51 cm; 3.5 − 0.23ka BP). The distinguishing feature of this part of the ANTU1+2 core is the presence of abundant aquatic moss fragments within the lake marts, particularly prior to ~1.2ka BP (Figs. 3,4). Scorpidium scorpioides is a calciphilous, brown, rich-fen moss that thrives in shallow water or on inundated peat bogs. It possesses special adaptations to prevent emergence and desiccation, favouring open, sunny habitats (Borysowski, 1978; Proctor and Smirnoff, 2000). The bulk characteristics of this core section are rather variable (CaCO₃ content: 54−86%; TOC content: 17−46%), reflecting increasing instability in the lake. C/N ratios (9−17) and biomarker indices ($P_{al}$: 0.35−0.87; $P_{aq}$: 0.47−0.73) are compatible with varying proportions of algal and moss detritus, since Scorpidium has an average C/N ratio of ~35 and $P_{aq}$ values ≥ 0.92 (Zibulski et al., 2017). Like other bryophytes, however, it does not produce a large amount of n-alkanes, although their distribution peaks at n-C₂₅ like Chara (Mead et al., 2005) and many submerged vascular plants (Ficken et al., 2000). Cyperaceae pollen, associated with sparse macrofossils of Carex and Typha, represents emergent aquatics. Coniferous forest (Pinus, Picea) dominated the regional landscape, together with Alnus and a declining percentage of Betula. Human impacts intensified after ~2ka BP (Laumets et al., 2014). Carbon-isotope values in this core segment edged up a little (TOC: −44 to −37‰; cellulose: −42 to −33‰), notably in the Pale laminated marl (134−51 cm; 1.2−0.23ka BP) (see Discussion). Bulk $\delta^{14}$C cellulose (11−19‰), moss $\delta^{13}$O cellulose (15−20‰), reconstructed $\delta^{18}$O water (−17 to −8‰) and estimated MAT values (−6 to +13°C), if taken at face value,
imply that the most pronounced warm period of the entire Holocene occurred between ~3.5 and 1.2ka BP (Fig. 6), which seems unlikely. We hypothesize that the marginal shallows and possible inundation of surrounding peatland recorded by Scorpidium resulted in warmer water temperatures and increased evaporation in summer, causing the isotopic composition of the lake to evolve along a local evaporative line (LEL), in contrast to the present day (Fig. 2c).

Marl (50–0 cm, 0.23ka BP - Present). Data for this interval are sparse, due to a shortage of material from surface core SURF and a lack of palaeoecological data. The five available samples only represent the uppermost 10 cm (the last few decades). Their TOC content ranged from 23 to 32% and their low C/N ratios (8–10) were typical of lacustrine plankton. \( \delta^{13}C_{\text{cellulose}} (3–7\text{%}) \) also deviated markedly from the rest of the core, implying implausibly low average lake-water temperatures (Fig. 6). These samples probably represent an algal flocc deposited during the early spring bloom, when the isotopic composition of the water may be strongly influenced by snowmelt (section 3.1), especially as the lake has become enriched with N and other nutrients in recent years (Saarse and Liiva, 1995).

4. Discussion

4.1. Changing cellulose sources

The \( \delta^{13}C \) values of cellulose and TOC were tightly coupled through the Holocene (\( R^2 = 0.80 \); Figs. 3, 4, 5a), notably during the more variable periods. Hence, our measured C/N ratios (<20) in bulk organic matter (Fig. 5b) and biomarker indices, supported by the available palaeoecological data, imply that aquatic-cellulose sources dominated the sediment record of Antu Sinijärve. Nevertheless, several contrasting biogeochemical phases can be identified (Fig. 5). From the base of the sequence (~11.4ka BP) until ~10.4ka BP, when marl deposition was initiated, the lake was probably at least 3–5 m deeper than today (Saarse and Liiva, 1995); aquatic microalgal and minor terrestrial organic-matter sources prevailed (Phase 1). From ~10.4ka BP onwards, aquatic productivity increased. The organic fraction mainly comprised a diverse mixture of aquatic inputs from green micro- and macroalgae (charophytes) and floating/submerged vascular plants, with a minor contribution from emergent macrophytes (Phase 2). Increasing paludification after ~6.6ka BP heralded the separation of Palaeolake Antu into the
three smaller modern lakes (Antu Sinijärve, Rohejärve and Valgejärve) by declining water levels and peat growth (Lehtmaa, 2006; Nedelskaia, 2011). Phase 3 (−3.5 to at least 0.23ka BP) was characterized by the deposition of abundant brown-moss macrofossils within the lake marls, in response to continued infilling by sediments and expansion of marginal peatlands. Data for the last 200–300 years (Phase 4) are sparse. However, the uppermost sediments appear to be of planktonic origin.

In addition to hydrothermal changes resulting from gradual infilling of the basin by lake sediments and marginal mires, the sediment record of Antu Sinijärve carries the imprint of major shifts in the biogeochemistry of surrounding terrestrial ecosystems. Phase 1 records the transition from the open landscape of the Preboreal to a birch-conifer forest. During Phase 2, the arrival of first, temperate and later, thermophilous deciduous tree species was accompanied by the development of a more diverse and nutrient-demanding lacustrine ecosystem including flourishing stands of benthic macroalgae (charophytes). After ~5.6ka BP, re-invasion by conifer (spruce-pine) forest, followed by human impact and paludification, began to reverse this trend, culminating in the creation of shallow and/or marginal habitats in which calciphilous brown mosses could thrive (Phase 3). Phase 4 appears to reflect recent cultural eutrophication (Saarre and Liiva, 1995).

4.2. Carbon- and oxygen-isotope records from Antu Sinijärve

4.2.1. Carbon isotopes

A remarkable feature of the δ13C records from Antu Sinijärve is the large decrease in the average δ13C values of both TOC and cellulose (by 10% and 14%, respectively) between Phase 1 and Phase 2 (Fig. 5a,c). More than half this change took place toward 10.4ka BP in core ANTU1+2, coinciding with the onset of biogenic calcification of the lake water, resulting in marl deposition (Fig. 5d). It represents a major shift in the entire lake-catchment carbon cycle. Similar (3–10%) decreases in the δ13C values of bulk organic matter and carbonates during the early Holocene in Sweden and Estonia were attributed by Hammarlund (1993), Hammarlund et al. (1997), Punning et al. (2000) and Stansell et al. (2017) to soil development associated with the transition from pioneer herb communities to subarctic birch and then boreal pine forests, resulting in an increased influx of 13C-depleted terrestrial biogenic carbon into the lakes from their catchments.

The 13C content of the dissolved organic carbon (DIC) pool in lakes may be far from equilibrium with the atmosphere. It is mainly controlled by: i) the δ13C value of the DIC in inflowing waters; ii) isotopic exchange with atmospheric CO2; iii) the photosynthetic and respiratory processes of aquatic macrophytes and algae; and iv) the decay of organic matter (Dana and Devey, 1960; Street-Perrott et al., 1993). Extremely 13C-depleted carbon-isotope values (−22 to −10% in DIC and −47 to −40% in plankton) were reported by Rau (1978) from a small, deep, oligotrophic lake surrounded by dense conifer forest on the Cascade Range, USA. Typical δ13C values for the forest biomass were −29 to −25%. He attributed the extraordinarily low δ13C values measured on plankton mainly to uptake of 13C-deficient respiratory CO2 released from catchment soils and in-washed terrestrial detritus. However, more fertile freshwater lakes like Antu Sinijärve, which receive substantial inputs of groundwater with DIC chemically and isotopically controlled by both respiration and reactions with aquifer carbonates, may also exhibit marked 13C disequilibrium with the atmosphere, as exemplified by Land and Epstein (1970); Street-Perrott et al. (1993) and Holmes et al. (1995), who reported 13C-depleted values between −13 and −5% for DIC in Jamaican river- and groundwater. Chemical weathering of limestone associated with high concentrations of soil CO2 transmits this depleted carbon-isotope signature of biogenic carbon from the catchments into surface waterbodies via the karst drainage system, resulting in lacustrine carbonates with isotope values as low as ~12‰.

Isotopic re-equilibration of the waters of small lakes with the atmosphere may be surprisingly inefficient (Street-Perrott et al., 1993; Holmes et al., 1995). However, had Antu Sinijärve ever become hydrologically closed during the Holocene, productivity-driven 13C-enrichment of the DIC, and the resulting organic and carbonate fractions, would probably have occurred (Talbot, 1990). Alternatively, Hammarlund et al. (2005) have suggested that oxidation of 13C-depleted CH4 generated in anoxic sediments lowered the 13C content of the DIC during the 8.2ka event in a Swedish marl lake. A requirement of the CH4 hypothesis as an explanation for very low δ13C values in lacustrine organic matter from Antu Sinijärve, however, is that the 13C-enriched co-genetic CO2 produced by methanogenesis (Herczeg, 1988) be effectively dissipated.

In the case of Antu Sinijärve, temporal changes in the carbon metabolism of the lake were closely coupled to the biogeochemistry of terrestrial ecosystems throughout the Holocene. At different stages, it was populated by a range of aquatic organisms with adaptations to different carbon sources and concentrations. During Phase 1, cold, subarctic conditions with pioneer vegetation and weakly developed soils (Punning et al., 2000) were associated with low counts of the slow-growing, green colonial alga Botryococcus. This has evolved several highly specialized strategies for coping with oligotrophic, C- or N-limited waters (Belcher, 1968; Ohmori et al., 1984; Cepák and Lukavský, 1994). It is able to assimilate HCO3−, the dominant DIC species in moderately alkaline lakes, as a C source. It also produces abundant oil droplets (isoprenoid alkyl ketones), which keep the algal colonies afloat at the water surface where light intensity and atmospheric CO2 influx are maximized.

The greatest limnic biodiversity occurred during Phase 2, registered by abundant fossils of charophytes, green microalgae and floating/submerged aquatics, implying a sustained influx of DIC and nutrients from the catchment, corresponding to the development of brown forest soils (Punning et al., 2000) under thermophilous deciduous forest. During this phase, very low δ13CTOC and δ13CCellulose Values below ~40% were persistently registered. A high proportion of the aquatic organisms recorded (apart from macrophytes with floating leaves, e.g. Nuphar, Nymphaea) are able to utilize bicarbonate as a C substrate (Maberly and Spence, 1983). The abundance of oogonia suggests that Chara stands were a particularly important cellulose source. This macroalga possesses sophisticated ion pumps that enable it to assimilate dissolved HCO3− (McConnaughey and Falk, 1991). For every two HCO3− ions taken up from the water, one is precipitated in the form of carbonate en masse (Street-Perrott et al., 2002), providing an additional 13C-deficient CO2 source during periods of marl formation (Hammarlund et al., 1997).

During ~5.6ka BP onwards, the supply of biogenic DIC and dissolved organic carbon (DOC) may have been maintained by the development of boreal conifer forest and peatland around the lake, augmented after ~5ka BP by human-induced land-use changes (deforestation and agriculture), which have been shown to increase the flux of DIC into lakes through the hydrological system (Maberly et al., 2013). Measurements by Bain and Proctor (1980) suggest that Scrophulidium scrophioides, like other aquatic bryophytes of hardwater lakes, is an obligate dissolved-CO2 user, thereby helping to perpetuate the low 13C values of TOC and cellulose observed in the
lake muds. No carbon-isotope analyses are available for the cellulose fraction of the surface sediments in core SURF, due to shortage of material.

4.2.2. Oxygen isotopes in cellulose and carbonate

The δ18Ocellulose record from the ANTU1+2 core (Figs. 3–4) exhibits both step changes attributable to local hydrological and ecological perturbations, and centennial-scale palaeoclimatic events that are discussed below in section 4.3. The pronounced isotopic shift in δ13C/δ18O space across the Phase 1–2 transition, −10.4ka BP (Fig. 5c) is accompanied by such a marked discontinuity in sediment properties (Fig. 5b,d) that a local hydrological change superimposed on more gradual processes like forest development and soil formation can be suspected. For example, a strong increase in groundwater discharge could have suddenly unblocked a veneer of glacial deposits that previously plugged the large spring vents (Saarse and Liiva, 1995), which now discharge HCO3−rich limestone groundwaters through the lake bed. Another event that may reflect local hydrological and hydroseral changes is the sharp rise in δ18Ocellulose values starting during the late Holocene (−3.1ka BP), early in Phase 3 (Figs. 3–4), which was attributed above to inundation of expanding marginal peatlands.

Several recent papers have made quantitative comparisons between δ18Ocellulose and δ18Ocarbonate in order to reconstruct lake-water temperatures by means of the so-called “calcite-cellulose geothermometer” (Rozanski et al., 2010; Heikkilä et al., 2010; Buhay et al., 2012). This involves using measured δ18Ocellulose values to calculate δ18Olakewater and then applying an empirical calcite-water equilibrium equation (usually Kim and O’Neil, 1997) to paired δ18Olakewater and δ18Ocarbonate values in order to calculate past changes in epilimnion temperature. This approach works well if the cellulose and calcite δ18O curves are broadly parallel. In Fig. 6, we compare the δ18Ocellulose record from core ANTU1+2 with the δ18Ocarbonate data from core ANTU3 ( Nedelskaja, 2011), supplemented by additional, unpublished analyses. Unfortunately, although these two curves vary largely in concert from ~9ka onwards, they diverge substantially between −11ka and ~9ka. This discrepancy is most evident from a comparison between our predicted δ18Ocarbonate curve for core ANTU1+2 and the δ18Ocarbonate measurements from core ANTU3 (Fig. 6). The maximum difference (Predicted-Actual) is −2.5‰. Buhay et al. (2012) also reported decoupling of carbonate δ18O and cellulose-inferred lake-water δ18O values in Deep Lake, Minnesota, between 11 and 9ka BP, in a not-dissimilar palaeogeographical situation on the southern side of the remnant Laurentide icesheet and the large, meltwater-fed proglacial Lake Agassiz.

Provided that the discrepancy between the Čntu Sinijärv δ18Ocellulose and δ18Ocarbonate records does not merely reflect unresolved stratigraphical or dating issues, or divergent limnological settings within different sub-basins of Palaeolake Čntu, it can be attributed to several types of “no-analogue” behaviour compared with the present day. First, it may not be valid to extrapolate the modern relationship between δ18Owater and air temperature, derived from the very short GNIP record, to reconstruct palaeotemperatures from the cellulose-based δ18Olakewater estimates (Fig. 6), especially beyond −9ka BP (cf. Guan et al., 2016; Tyler et al., 2016). Furthermore, the expanded Palaeolake Čntu may have evolved along a LEL during the dry early Holocene, without ever becoming hydrologically closed (cf. modern Estonian lakes sampled by Stainsell et al., 2017).

Secondly, past reconstructed variations in cellulose sources (section 4.1) suggest that the δ18Ocellulose signal may represent different seasons and environments of cellulose synthesis at different times. Algal blooms may have captured the low isotopic values of spring snowmelt to a greater degree during Phase 1 and the early part of Phase 2 than during the mid-Holocene (cf. Wolfe and Edwards, 1997). In contrast, after ~9ka, co-production of cellulose and calcite within dense Chara beds may have occurred predominantly during warmer conditions in summer and early autumn. Significant kinetic effects may also have occurred during rapid calcite precipitation (Coplen, 2007; Rozanski et al., 2010).

Thirdly, the regional climate and palaeogeography of Scandinavia and the Baltic Basin were markedly different from today during the interval −11−9ka BP. Orbital forcing and seasonal insolation anomalies were close to their maximum post-glacial values. The rapidly shrinking Fennoscandian and Laurentide Ice Sheets, major oceanographic changes in the North Atlantic and the impact of δ18O-depleted glacial meltwater on the greatly expanded predecessors of the modern Baltic Sea (section 2.1.1) would also have significantly affected seasonal airflow patterns (Mitchell et al., 1988; Renssen and Isarin, 2001; Tindall and Valdes, 2011) as well as the proximity, availability and isotope values of regional moisture sources (Heikkilä et al., 2010). Estimates of summer temperatures in Estonia based on rapidly responding proxies such as aquatic macrofossils (Valiranta et al., 2015) and chironomids (Heiri et al., 2014) suggest conditions ≤2°C warmer than today during the early Holocene. However, pollen-based reconstructions of Estonian summer and winter temperatures are bedevilled by very low pollen counts and by the delayed arrival of forest trees from their glacial refugia (Valiranta et al., 2015; Veski et al., 2015). The development of fully self-consistent, climatic, hydrological and isotopic scenarios for the early Holocene needs to draw on the power of isotope-enabled GCMs to simulate regional anomalies throughout the entire annual cycle in response to past changes in orbital forcing and climatic-boundary conditions (cf. LeGrande and Schmidt 2008; Daley et al., 2011; Tindall and Valdes, 2011; Liu et al., 2014; Holmes et al., 2016).

4.3. Centennial-scale palaeoclimatic events

Three δ18O minima of wide palaeoclimatic significance are recorded in the Čntu Sinijärv cellulose sequence: the PBO, the 8.2ka event and an unnamed event centred on −3.25ka BP (Fig. 6). The first two have been linked to oceanic impacts of meltwater discharges from the decaying Laurentide and Fennoscandian Ice Sheets (Björck et al., 1996; Fisher et al., 2002; von Grafenstein et al., 1998, 1999; Hammarlund et al., 2005; Daley et al., 2011; Tindall and Valdes, 2011), whereas the third has an uncertain cause. It is one of a series of mid-Holocene climatic oscillations recorded in European bogs and lakes (Barber et al., 2004), but is particularly pronounced in Čntu Sinijärv.

The PBO is recorded by the deep δ18O minimum (reconstructed δ18Olakewater −21.06 to −20.97‰) at the base of the Čntu Sinijärv sequence, dated −11.3−11.2ka (Fig. 6a). Although these values are similar to present-day February snowfall, both they and the mean-annual palaeotemperatures derived from them (−14.1 to −13.9°C) seem implausibly low, given that the TOC content of the sediment is minimal, the dominant carbon source was microalgae, which may have adopted the isotopic signature of spring snowmelt, and the available GNIP record used to calibrate the lakewater δ18O values is uncomfortably short; hence, the slope of the MAT/δ18O regression line is not well constrained.

The ANTU1 core confirms that δ18Ocellulose has the potential in principle to provide a detailed record of the 8.2ka cold event (Fig. 6b). It displays double isotope minima at −8.39 and −8.26‰ BP, with reconstructed δ18Olakewater values of −16.4 and −15.1‰, corresponding to estimated annual MATs of −4.4 and −1.6°C, respectively, followed by a warm “overshoot” to −11.9°C at −8.17ka (which has been identified by Andersen et al. (2017) in several other δ18O records ranging from Greenland to Austria). Although
many high-resolution records of the 8.2ka event from around the North Atlantic also exhibit double isotope minima, there is as yet little consensus about the timing of the two events, in part due to dating issues (Daley et al., 2011). Unfortunately, however, not only does the amplitude of isotopic variation in core ANTU1 seem rather large in comparison with both more proximal and more distal sites (von Grafenstein et al., 1998; Daley et al., 2011; Baker et al., 2017), but the ANTU2 core displays a much more muted signal than ANTU1 in the region of overlap (Fig. 6b), making it difficult to draw firm palaeoecological conclusions from this part of the $\delta^{18}O_{\text{cellulose}}$ record.

The 3.25ka $\delta^{18}O$ minimum lasted for ~300 years, from ~3.4 to ~3.1ka BP (Fig. 6a). $\delta^{13}C_{\text{cellulose}}$ reached a low of $+11.3\%$, implying a $\delta^{18}O_{\text{lakewater}}$ value of $-16.3\%$ and an annual MAT around $-4.0\, ^\circ\text{C}$, similar to the modern January mean in Tartu (Fig. 2a). A search for other palaeoecological records from eastern Europe that include this cold event would help to determine its wider significance.

4.4. Cellulose isotope records as palaeoenvironmental indicators in a shallow, hardwater lake

Hardwater lakes offer significant advantages for cellulose-isotope studies. They are often more productive and biodiverse than softwater lakes, leading to sediments rich in cellulose, which provides a standard chemical material for isotope analysis, unlike TOC. $\delta^{13}C_{\text{cellulose}}$ and $\delta^{18}O_{\text{cellulose}}$ analyses can be coupled with isotope measurements on endogenic and biogenic carbonates or biogenic silica from the same core levels, thereby offering detailed insights into past changes in hydrology and carbon cycling, not just on a whole-lake basis but also with a prospect of resolving different trophic levels, habitats and seasons. Given ideal conditions, cellulose-calcite and cellulose-silica geothermometry can be attempted (Rozanski et al., 2010; Heikilä et al., 2010; Buhay et al., 2012). In Antu Sinijärv, shifts in $\delta^{13}C_{\text{cellulose}}$ values were related to major events affecting the carbon cycle in the coupled lake-catchment ecosystem. This study has also demonstrated that with a soundly based chronology, major abrupt palaeoclimatic events can be identified within a century or so of their accepted dates of occurrence, although their isotopic magnitude and estimated palaeotemperature implications may be exaggerated in this sequence due to past departures from observed seasonal, hydrological and isotopic behaviour resulting from orbital forcing and changing climatic-boundary conditions (section 4.2.2). The signature of local and regional hydrological events such as lake-level variations and hydroseral succession has also been detected in the $\delta^{18}O_{\text{cellulose}}$ record.

However, the diverse range of aquatic cellulose sources prevalent in a hardwater lake (microalgae, macroalgae, calciphilous bryophytes and vascular plants), not to mention potential “terrestrial” sources (emergent macrophytes and land vegetation that use atmospheric CO$_2$) could be viewed as a disadvantage. With some success, but also with significant effort, this study has identified past changes in major cellulose sources with the aid of C/N ratios and biomarker indices calculated from GC-MS analyses of the solvent extracts remaining after cellulose preparation, together with several types of palaeoecological data (pollen, plant macrofossils and other microfossils) that are available for earlier cores from the same lake. The rich plant-physiological literature on aquatic plants and algae provides a valuable resource for understanding the carbon metabolism of the major cellulose producers identified in this way, although more detailed work on non-pollen palynomorphs could well lead to surprises.

The temperature-independent oxygen-isotope fractionation between source water and cellulose is an important advantage of cellulose-isotope analysis, as noted in the Introduction. However, the greater the degree of water-column stratification, variability of dominant water sources (rainfall, ground water, snowmelt), diversity of microhabitats or seasonality of cellulose production by different organisms, the greater the need for isotope analyses of modern plants and waters in order to resolve the resulting complexity. Fortunately, the present-day Antu Sinijärv is an open lake with a short water-residence time that only displays very weak oxygen-isotope stratification. This study has attempted to estimate Holocene palaeotemperatures using monthly weighted-average $\delta^{18}O_{\text{water}}$ and temperature data from the nearest GNIP station; however, the results should be treated with caution, as noted in section 4.2.2. It is also important to recognize that submerged vascular plants and Chara living on the bed of the lake are probably bathed in $^{18}$C- and $^{13}$C-depleted spring waters throughout their respective growing seasons (cf. Sensula et al., 2006). Unfortunately, only one relevant plant analysis from Antu Sinijärv (Ceratophyllum demersum: $\Delta^{14}C$ on carbonate encrustations $-147 \pm 6.7\%$ in 1970; $\delta^{13}C_{\text{COC}}$ $-35\%$; Olsson and Kaup, 2001) and no $\delta^{13}C$ analyses of the DIC are available to gauge the magnitude of groundwater impacts.

The limited $\Delta^{14}C$ data for Antu Sinijärv nevertheless confirm another well-recognized issue with the sediments of hardwater lakes, amply illustrated by Ralska-Jasiewiczowa et al. (1998), Olsson and Kaup (2001) and Sensula et al. (2006); namely, the need to base radiocarbon chronologies exclusively on recognizable terrestrial macrofossils such as seeds, twigs, conifer needles or bud scales (Nedelskaja, 2011), rigorously avoiding TOC, carbonates, aquatic macrofossils and amorphous terrestrial materials such as peat, all of which may display significant radiocarbon reservoir effects. However, the palaeoecological comparisons made in section 4.3 suggest that the Antu Sinijärv chronology used in this study, which was based exclusively on terrestrial macrofossils (Supplementary Data) stands up very well in this respect.

5. Conclusions

1. Shallow hardwater lakes have significant advantages for cellulose-isotope analysis, including biodiverse ecosystems that produce abundant cellulose, which can be analysed in parallel with lacustrine carbonates in order to achieve a more multidimensional perspective on past limnological changes.

2. Particular attention must be paid to the need for modern isotope data, supporting geochemical analyses and detailed palaeoecological records in order to resolve past shifts in dominant cellulose sources.

3. In this study, C/N ratios and biomarker indices were successfully employed in conjunction with data on pollen, plant macrofossils and Chara oogonia to reconstruct Holocene changes in the origin of the cellulose fraction.

4. 14C chronologies based exclusively on terrestrial macrofossils are essential in order to avoid serious reservoir effects common to hardwater lakes.

5. The Antu Sinijärv record described here begins in the Preboreal, ~11.4ka BP. The dominant sources of organic matter were aquatic throughout. The sequence can be divided into four main biogeochemical phases distinguished by significant cellulose inputs from microalgae (~11.4–10.4ka), charophytes (~10.4–3.5ka), bryophytes (~3.5 to at least 0.23ka) and microalgae (the last few decades), respectively.

6. The $\delta^{13}C_{\text{cellulose}}$ record from Antu Sinijärv exhibits major variations that agree well in timing with published $\delta^{13}C_{\text{carbonate}}$ curves but are significantly larger in amplitude. Throughout the Holocene, temporal changes in the carbon metabolism of the lake were closely coupled to the biogeochemistry of terrestrial ecosystems.
7. The δ18Ocellulose record displays both step changes of probable local hydrological origin, and major centennial-scale cold events of wide palaeoclimatic significance (the Preboreal Oscillation, the 8.2ka event and an unnamed event at ~3.25ka), the ages of which correspond closely in timing with published syntheses.

8. The δ18Ocellulose and δ13Ccarbonate curves for Palaeolake Antu are in good agreement though the period of overlap after ~9ka BP. However, during the “no-analogue” interval between ~11ka and ~9ka they diverge markedly. At that time, the oaribly forced increase in Northern-Hemisphere seasonality, combined with rapidly changing North Atlantic palaeoceanography and substantially altered regional palaeogeography, probably resulted in isotopic relationships and seasonal patterns of cellulose and carbonate synthesis that deviated markedly from modern observations.

Acknowledgments

Funding for this study was provided by the UK NERC Rapid Climate Change Consortium (NER/T/S/2002/00460, NE/F011527/1). We thank Nathan Peter Scott for his conscientious assistance with literature review, laboratory analyses and data compilation.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.quascirev.2018.05.010.

References


Sensula, B., Böttger, T., Pazdur, A., Piotrowska, N., Wagner, R., 2006. Carbon and oxygen isotope composition of organic matter and carbonates in recent lacus-


Sohar, K., Kalm, V., 2008. A 12.8-ka-long palaeoenvironmental record revealed by subfossil ostracod data from lacustrine freshwater tufa in Lake Sinijärv, north-


Sternberg, L. 1989. Oxygen and hydrogen isotope ratios in plant cellulose: mechan-


Tindall, J.C., Valdes, P.J., 2011. Modeling the 8.2 ka event using a coupled
line, North-Central Russia, inferred from oxygen isotope records in lake sedi-

Wolfe, B.B., Edwards, T.W.D., Teuling, H.C., 2000a. A 6000-year record of interaction of paleohydrology and paleoclimate at tree-
line. North-Central Russia, inferred from oxygen isotope records in lake sedi-


ments: Physical and Chemical Techniques, Developments in Paleoenviron-

yology using lake sediment cellulose. J. Paleolimnol. 37, 221–231.


Yakir, D., 1992. Variations in the natural abundance of oxygen-18 and deuterium in precipitation over the central United States: implications for the use of surface sediment calibrations in paleo-