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Molecular fossils as a tool for tracking Holocene sea-level change in the Loch of Stenness, Orkney

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ABSTRACT: Sediments deposited in the Loch of Stenness (Orkney Islands, Scotland) during the Holocene transgression, previously dated to between ~5939–5612 BP, were analysed for molecular fossils – lipids and chlorophyll pigments from primary producers – that complement conventional microfossil and lithological approaches for studying past sea-level change. While microfossil and lithological studies identified a transgression between 102 and 81 cm core depth, key molecular fossils fluctuate in occurrence and concentration between 118 and 85 cm, suggesting an earlier start to the transgression. Terrestrial lipid concentrations decreased and algal-derived, short-chain, n-alkanoic acid concentrations increased at 118 cm, indicating a disruption of the freshwater lake conditions associated with the early stages of the marine transgression. The lipid and pigment analyses provided information that complements and extends that from microfossil analysis, presenting a more complete record of Holocene sea-level changes and local vegetation changes in the Loch of Stenness. The isostatic stability of Stenness during the Holocene points towards other factors to explain the transgression, such as regional factors and/or melting of the Antarctic ice sheet (which occurred up to 3 ka).

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KEYWORDS: chlorophylls; Holocene; lipids; molecular fossils; sea level

Introduction

Changes in sea level have been studied extensively to understand their causes and impacts on the environment and human populations (Rollins et al., 1979; Hodgson et al., 2009; Bates et al., 2016; IPCC, 2019). By understanding the main drivers and consequences of past sea-level changes, this knowledge can be applied to understanding future climate and sea-level change scenarios on local and global scales (Church et al., 2008).

Transgressions and regressions can lead to changes in the dominance of terrestrial or marine sources of organic matter (OM) at a particular location. Nutrient availability, degradation of OM and sedimentation rates can also be affected to different extents, strongly depending on the basin topography. The changes in water depth inherent in a transgression may lead to changes in sedimentation and in the extent of sediment reworking (Cattaneo and Steel, 2003). During a regression, nutrient input may decrease, thus reducing productivity and sedimentation of OM (Cattaneo and Steel, 2003). Localized fluctuations between terrestrial- and marine-dominated conditions in coastal/near coastal regions can be inferred from seismic mapping, palaeontological analysis, changes in the nature and morphology of the sediments, and changes in the composition and nature of OM (Shennan et al., 2015). Environmental proxies for tracking transgressions based on soil and sediment analysis exploit shifts in the populations of plant macrofossils, insects, pollen and spores, foraminifera, diatoms, molluscs and ostracods (Horton et al., 1992; Bunting, 1994; Davis et al., 2003; Bates et al., 2016).

While a major challenge in using macro- and microfossil evidence is their extent of preservation and/or limited number of specimens, it is often possible to extract organic geochemical molecular fossils incorporated into the sediment matrix. Through their relationship to the biological molecules from which they originate, the molecular fossils can reflect specific OM inputs (Fig. 1) and thereby reveal characteristics of, and changes in, the environment inhabited by the source organisms (Poynter and Eglinton, 1990; Meyers and Ishiwatari, 1993; Castañeda and Schouten, 2011). Molecular fossils can be analysed within milligram to gram quantities of sediment, a much smaller scale than the few grams needed for traditional macro- and microfossil analyses (Shennan et al., 2015). Hence, sampling resolution can be reduced, even to millimetre scale where justifiable, enabling analyses at higher temporal resolution (Airs and Keely, 2002). Robust lithological and morphological studies are key to identifying the areas of undisturbed sediments that are ideal for palaeoenvironmental and molecular fossil analyses, avoiding compromised areas (e.g. where bioturbation or burrowing has occurred).

Molecular fossils inferring sea-level changes

The widespread occurrence of molecular fossils in sediments and their derivation from both aquatic and terrestrial sources allows the recognition of allochthonous and autochthonous sources of OM in sediments (Eglinton and Hamilton, 1967; Cranwell, 1982; Keely, 2006). Extensive research on the diagenetic transformation of various biomarkers enables reconstructions based on the principal molecular fossils of
primary producers, such as chlorophyll pigments, n-alkanes, alcohols, fatty acids and tetraether lipids (Eglinton and Hamilton, 1967; Harradine et al., 1996; Ais et al., 2001; Volkman, 2005; Schouten et al., 2013). However, the use of molecular fossils to identify and study sea-level fluctuations has been largely overlooked with only a few studies reported in the literature (e.g. Ficken and Farrimond, 1995; Squier et al., 2002; Bendle et al., 2009; van Soelen et al., 2010; Carr et al., 2015; De Jonge et al., 2016; Li et al., 2017). The combined use of several molecular fossils is key to identifying sea-level changes (Fig. 1). It can be expected that a transgression will result in a shift in the molecular fossil assemblage from mostly terrestrial OM (from either land runoff or in situ production) to a more significant marine contribution (Fig. 1).

Terrestrial OM reflects the vegetation in the hinterland through the distributions of: sterols that occur widely in higher plants (Volkman, 1986), long odd-chain n-alkanes (n-C25–32) and long even-chain n-alkanols and n-alkanoic acids (n-C22–30) prominent in higher plant waxes (Cranwell, 1973, 1982). Furthermore, branched glycerol dialkyl glycerol tetraether lipids (iGDGTs), structural cores of the polar membrane lipids of eubacteria, represent soil microbial communities (Fig. 1; Schouten et al., 2013). By contrast, aquatic conditions are represented by isoprenoid GDGTs (iGDGTs), derivatives of the structural membrane lipids of commonly occurring Archaea (Schouten et al., 2013). Among these, crenarchaeol is a specific marker for marine Archaea (Damsté et al., 2002). Although low level production of iGDGTs in soil and some brGDGTs in aquatic environments has been noted (DeLong, 1992; DeLong et al., 1994; Hershberger et al., 1996; Tierney and Russell, 2009; Fietz et al., 2012; Li et al., 2016), the general source distinction between the two groups is employed widely for tracing the origin and transport patterns of soil OM (Sun et al., 2011; Doğru Selver et al., 2012; Zhu et al., 2013). Other markers of aquatic conditions include short-chain-length n-alkanes (n-C17–24), common in bacteria and algae (Cranwell, 1973), and predominance of short even-chain n-alkanols and n-alkanoic acids (n-C16, n-C18), although these also occur in some plants (Meyers and Ishiwatari, 1993). Long-chain unsaturated alkenones (n-C27–30) are common markers of haptophyte algae in marine sediments, especially C27:3 and C27:2 (Markewit et al., 1984). In coastal as well as freshwater and saline lake systems, C27:4 occurs alongside C27:2 and C27:3 (Cranwell, 1985; Bendle et al., 2009; Table 1).

Changes in molecular fossil distribution correlate with changes in environmental conditions, giving rise to proxies that reflect the origins of OM, water temperature and soil pH. Specific proxies, indicators of palaeoenvironmental conditions, have been suggested to distinguish inputs of plant OM (average chain length, ACL; Poynter and Eglinton, 1990), terrestrial from marine OM based on brGDGTs and crenarchaeol (branched and isoprenoid tetraether index, BIT; Hopmans et al., 2004), and terrestrial from freshwater OM based on n-alkane carbon chain lengths (proxy of aquatic macromolecules, PwF; Ficken et al., 2000; Table 1). In immature sediments, the carbon preference index (CPI; Bray and Evans, 1961) can reflect predominance of either terrestrial or algal OM (Clark and Blumer, 1967; Ortiz et al., 2004; Table 1). The tetraether index of 86 carbons (TEX86; Schouten et al., 2002; Table 1), based on iGDGTs, is a proxy for sea-surface water temperature (SSS), and mean annual air temperature (MAAT) can be reconstructed from the methylation of branched tetraethers (MBT; Weijers et al., 2007). A proxy proposed to reconstruct soil pH is based on brGDGTs (cyclization of branched tetraethers, CBT; Weijers et al., 2007; Table 1). Other proxies for temperature estimations exist but are not applied in this work; for example, U37Cδ is based on long-chain alkenones (Brassell et al., 1986; Prah and Wakeham, 1987) and RAN15, RAN13 and RAN17 on 3-OH fatty acids (Wang et al., 2016; Yang et al., 2020).

Changes in the availability of nutrients in aquatic environments can be reflected by fluctuations in the concentrations of particular chlorophyll pigments (broadly described as chlorins) produced by the photosynthetic organisms inhabiting the water column (Fig. 1; Keely, 2006). The physicochemical conditions in the water column are reflected by particular pigment structures. Photoautotrophs that live in oxygen-rich waters, including aquatic plants, photosynthetic algae and cyanobacteria, produce chlorophyll pigments (chl; Scheer, 1991). By contrast, bacteriochlorophylls (bchls) are the photoreceptors in the purple and green bacteria, which are restricted to anoxic waters (Fig. 1; Plenning, 1977). Covariance in organic carbon and chlorin accumulation provides evidence that pigment concentrations reflect changes in productivity, for example, during glacial/interglacial cycles (Harris et al., 1996). Short-term transgressions can be evident in changes in the nature and distribution of chlorophyll pigments (Squier et al., 2002; Hodgson et al., 2009).

**Aims**

There is considerable potential for routinely using molecular fossils for sea-level studies. In the UK, Holocene transgressive sequences have been extensively studied in many of the larger estuaries and coastal environments in England (Devoy, 1977; Long et al., 2000; Sidell, 2003; Bates and Stafford, 2013) and Scotland (Long et al., 2016; Palamakumbura, 2018). In most cases, however, the precise nature and duration of the flooding
## Materials and methods

### Site description

The Loch of Stenness is an ~4-km-long brackish lake situated in the main island of the Orkney archipelago, Scotland (Fig. 2a), and connected to the sea at the Brig O’Wattie (Fig. 2b). The area has been inhabited since at least 3500 BC (~5.5 ka); the extensive archaeological evidence forms part of the Heart of Neolithic Orkney World Heritage Site (Farrell et al., 2014).

### Previous studies of the Loch of Stenness Core 2014–1

The core lithology (Core 2014-1; Fig. 2c) consists of a grey–brown organic silt with mollusc shells between 200 and 141 cm core depth, overlain by a pale grey silt with occasional shell fragments (141–81.5 cm). The uppermost sediments (81.5–0 cm) comprise soft, dark grey silt with occasional shell fragments of brackish species. The loss-on-ignition profile (Fig. 3) indicates that the organic component was >20% below 140 cm depth and between 10 and 17% above 140 cm depth; higher values are associated with the coarsest sediments of the core. Carbonate-rich sediments below 85.5 cm (10–26%) and seismic layering are associated with slower sedimentation and controlled deposition in a freshwater environment (Bates et al., 2016).

Palaeoenvironmental reconstruction, inferred from foraminifera, mollusc and ostracod assemblages, suggests two main environmental settings: a freshwater coastal lake (200 and 104 cm) and a brackish loch (102 and 20 cm). The brackish environment was further subdivided on the basis of the microfossil fauna: 102–81 cm shows onset of tidal access under very low brackish conditions (mix of brackish and freshwater ostracods) and 80–0 cm represents a brackish environment in which decalcification occurred and a restricted foraminiferal assemblage existed (Supporting Information, Table S1; Bates et al., 2016).

Radiocarbon dating of *Lymnaea* sp. shells at 92–94 cm and at 82–84 cm showed that the incursion began between ~5939 and 5753 BP and was fully established between ~5862 and 5612 BP (Table S2; Bates et al., 2016).

### Table 1. Proxies developed from biological markers of terrestrial, freshwater and marine organic matter (OM).

| Proxy | What it represents | Markers | Values and inference | References |
|-------|-------------------|---------|---------------------|------------|
| Branched and isoprenoid tetraethers (CBT) | Lake temperature | Tetraethers index of 86 carbon atoms | Reef surface temperature iGDGTs, including crenarchaeol | Schouten et al. (2002) |
| Branched and isoprenoid tetraethers (CBT) | Reconstruction of soil pH | Lake-water temperature in °C | Lake-water temperature in °C | Pearson et al. (2011) |
| Branched and isoprenoid tetraethers (CBT) | Reconstruction of sea-surface temperature | Sea-surface water temperature in °C | Sea-surface water temperature in °C | Pearson et al. (2011) |
| Branched and isoprenoid tetraethers (CBT) | Lake water temperature | Lake-water temperature in °C | Lake-water temperature in °C | Pearson et al. (2011) |
| Branched and isoprenoid tetraethers (CBT) | Reconstruction of soil pH | Lake-water temperature in °C | Lake-water temperature in °C | Pearson et al. (2011) |
| Branched and isoprenoid tetraethers (CBT) | Reconstruction of sea-surface temperature | Sea-surface water temperature in °C | Sea-surface water temperature in °C | Pearson et al. (2011) |
| Branched and isoprenoid tetraethers (CBT) | Lake water temperature | Lake-water temperature in °C | Lake-water temperature in °C | Pearson et al. (2011) |
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| Branched and isoprenoid tetraethers (CBT) | Reconstruction of soil pH | Lake-water temperature in °C | Lake-water temperature in °C | Pearson et al. (2011) |
| Branched and isoprenoid tetraethers (CBT) | Reconstruction of sea-surface temperature | Sea-surface water temperature in °C | Sea-surface water temperature in °C | Pearson et al. (2011) |

 event remains difficult to determine. Sea-level index points from Scottish sites show sea-level fluctuations (~12–3 ka) resulting from glacio-eustasy and isostasy, subsequently approaching present-day levels (~3 ka to present; Shennan, 1989; Shennan and Horton, 2002; Selby and Smith, 2007). The timing of the fluctuations in the Mid-Late Holocene varies regionally; for example, Orkney sea-level approached present values ~5–4 ka BP (Shennan and Horton, 2002). Seismic and palaeoenvironmental analyses of a series of LateGlacial to Holocene sediment cores from the Loch of Stenness, Orkney, demonstrated the impact of the transgression on the local geography and how it might have impacted human settlement (Bates et al., 2016).

Building on the existing research on sea-level change at the Loch of Stenness, the overall aim of this study was to use this well-constrained transgression record to develop a complementary method to study sea-level change using molecular fossils, largely overlooked in sea-level studies. Analysis of a combination of molecular fossils could provide a valuable record of marine and terrestrial OM inputs and changes associated with the transgression. This approach has the potential to provide more detailed and explicit environmental records of the impact of the transgression on sedimentary OM, allowing better constraints on the timing of events.
Separation of mollusc shells

The freeze-dried sediment was inspected for intact gastropod shells before grinding and sieving. Any shells found were removed with a spatula and/or forceps and sonicated in deionized water to remove sediment residues. The species were identified after air-drying in a fume hood overnight.

Extraction and analysis of molecular fossils

For complete methods, see Table S3. The core was subsampled at 10 depths (Fig. 3). The freeze-dried sediment was homogenized and sieved (Saesaengseerung, 2013). Extraction of OM from this fraction was carried out using accelerated solvent extraction (Schouten et al., 2007; Saesaengseer-

Figure 2. (a) Map of the British Isles showing the Orkney archipelago (white square) and the Loch of Stenness (green square; adapted from Google Maps); (b) map of the Loch of Stenness showing the Brigh O’Waite and the connection to the sea (adapted from Google Maps); (c) map of the Loch of Stenness showing the location of Core 2014-1 in the red rectangle. Bathymetry only shown for the Loch of Stenness (adapted from Bates et al., 2016). [Color figure can be viewed at wileyonlinelibrary.com]

Figure 3. Stenness Core 2014-1 showing the main lithological zones, the loss-on-ignition results (sampled every 5 cm) and the dates of the beginning and end of transgression according to Bates et al. (2016); sampling depths analysed in this study are marked with arrows. [Color figure can be viewed at wileyonlinelibrary.com]
The lipids were separated into four distinct polarity fractions using flash column chromatography (Green, 2013). These fractions were analysed by high-performance liquid chromatography with mass spectrometry (HPLC-MS of GDGTs; Schouten et al., 2007) and by gas chromatography-flame ionization detection (GC-FID) and selected samples by GC with mass spectrometry (GC-MS; Green, 2013). Following extraction, chlorophyll pigments were analysed by ultra-high performance liquid chromatography with diode array detector (UHPLC-DAD) and UHPLC-MS (Saesaengseerung, 2013).

**Results and discussion**

Shells of the freshwater gastropods *Gyraulus crista*, *Gyraulus laevis* and *Radix balthica* were identified between 197 and 85 cm depth, supporting the findings of Bates et al. (2016) who identified predominantly freshwater ostracods with some brackish species between 200 and 81 cm (Table 2). The following sections build on the interpretation of Bates et al. (2016), that the core sequence represents a freshwater lake environment in the lowest part of the core (Figs 4 and 5a; Table 3). The identification of iGDGTs (solid line), brGDGTs (dashed line) and crenarchaeol at 38 cm. Notably, at 118 cm there is a change in the OM: algal markers become the dominant components, reflecting a relative reduction in terrestrial deposition and increase in algal OM production, possibly enhanced by increased preservation resulting from water column anoxia (Fig. 6a,b,d). This observation probably indicates the disruption of the freshwater lake conditions associated with the early stages of the marine transgression identified from the microfossil assemblage at 102 cm (Bates et al., 2016).

Detail on the water column conditions was provided by the chlorophyll and bchl pigments. As is commonly observed, intact and early diagenetic transformation products (formed via enzymatic, herbivore grazing and oxidative processes) were incorporated into the sediment (Fig. S1; Shuman and Lorenzen, 1975; Hendry et al., 1987; Harradine et al., 1996; Ma and Dolphin, 1996; Walker et al., 2002; Keely, 2006). The fluctuations in the summed concentrations of chl and bchl pigments implies short-term changes in primary production (Fig. 6d). At 197 cm the predominance of chl pigments indicates a fully oxidized water column. The presence of bacterioviridin a (bvir a), an oxidation product of bchl a (Wilson et al., 2004), suggests either a shallow-water environment with significant fluctuations in oxygenation or that desiccation occurred at this time. Core depths between 182 and 118 cm record a mixed primary producer community with the dominant oxygenic population separated from a deeper anoxic population by a chemocline (Pfennig, 1977; Gervais, 1998). Chemoclines form in zones of oxygen depletion and reducing environments (Pfennig, 1977). The presence of a fully developed chemocline and absence of bvir a from 182 to 118 cm indicates that the OM was not affected by fluctuations in oxygenation, suggesting that the water column was deeper than at 197 cm.

### The freshwater lake (200–104 cm)

The predominance of brGDGTs and absence of marine marker crenarchaeol confirmed a freshwater lake environment in the lowest part of the core (Figs 4 and 5a; Table 3), with a prevalence of terrestrial OM input from runoff. The input of terrestrial OM was revealed by the occurrence of long odd-chain n-alkanes (Fig. 6a) and high CPI values (Fig. 5b; Table 3). The prevalence of terrestrial OM was further indicated by a dominance of long even-chain n-alkanols and n-alkanoic acids (Fig. 6b; Cranwell, 1982; Meyers and Ishiwatari, 1993), long odd-chain n-alkanes (Fig. 6a; Bray and Evans, 1961; Cranwell, 1973) together with stigmasterol and β-sitosterol, the major sterols of higher plants (the latter also an abundant component in emergent water plants; Fig. 6c; Meyers and Ishiwatari, 1993). Further evidence of terrestrial input comes from the presence of the sterol obtusifoliol of *Sphagnum* moss and vascular plant origins (Fig. 6c; Ronkainen et al., 2013). Although brGDGTs can have terrestrial and minor aquatic sources, the dominance of other plant/terrestrial markers suggests input from soils. Mixed terrestrial and emergent macrophyte origins for the n-alkanes, consistent with a permanent body of water during the freshwater stage, was evident by the occurrence of markers both for higher plant waxes and for freshwater macrophytes, reflected in the P<sub>aq</sub> index values (Fig. 5c; Table 3; Ficken et al., 2000). Evidence for an aquatic OM contribution from Archaea, algae and/or bacteria comes from small amounts of iGDGTs (Fig. 4; Powers et al., 2010; Bischoff et al., 2016; De Jonge et al., 2016) and from short-chain n-alkanoic acids occurring above 182 cm (Fig. 6b; Cranwell, 1982).
Figure 5. Lithology and radiocarbon dating from Bates et al. (2016), and proxies applied to the Loch of Stenness sediments: (a) BIT (branched and isoprenoid tetraether lipids); (b) CPI (carbon preference index based on n-alkanes); (c) $P_{aq}$ (proxy of aquatic macrophytes based on n-alkanes); (d) ACL (average chain length based on n-alkanes); (e) $pH$ calculated from CBT (cyclization of branched tetraether lipids); (f) $TEX_{86}^{L}$ values (tetraether index of 86 carbons – low) for sea-surface temperature calculations. [Color figure can be viewed at wileyonlinelibrary.com]

Table 3. CPI, $P_{aq}$, ACL and BIT indices, pigment and sterol evidence, $TEX_{86}^{L}$ values and sea surface temperatures (SST) calculated from $TEX_{86}^{L}$, and mean summer lake temperature for the Loch of Stenness sediments.

| Depth (cm) | CPI terrestrial vs. algal | $P_{aq}$ terrestrial vs. freshwater | ACL* grass vs. forest | BIT* terrestrial vs marine | Pigments | Sterols | $TEX_{86}^{L}$ values* | SST from $TEX_{86}^{L}$ (°C ± 4.0)* | Summer lake water temperature* (°C ± 2.1)* |
|-----------|---------------------------|-------------------------------------|-----------------------|----------------------------|----------|--------|----------------------|----------------------------------|---------------------------------------------|
| 14        | 13.3                      | 0.32                                | 29.0                  | 0.71                        | n.d.     | 0.55   | Low production       | -0.54                            | 18.6                           |
| 38        | 9.20                      | 0.25                                | 29.3                  | 0.62                        | n.d.     | 0.51   |                    | 13.1                             |                               |
| 77        | n.d.                      | n.d.                                | n.d.                  | 0.55                        | n.d.     | 0.50   |                    | 12.8                             |                               |
| 85        | 4.39                      | 0.15                                | 29.5                  | 0.96                        | Oxic     | 0.53   |                    |                                  |                               |
| 92        | n.d.                      | n.d.                                | n.d.                  | 1                          | Low production | n.d.  |                    |                                  |                               |
| 118       | 7.85                      | 0.25                                | 29.1                  | 1                          | Chemocline | n.d.  |                    | -0.49                            | 21.0                           |
| 155       | 11.3                      | 0.33                                | 28.4                  | 1                          |          | 0.54   |                    | 21.4                             |                               |
| 165       | 8.09                      | 0.32                                | 28.6                  | 1                          |          | 0.51   |                    | 20.5                             |                               |
| 182       | 6.23                      | 0.27                                | 28.4                  | 1                          | Shallow water | -0.55 |                    |                                  | 21.4                           |
| 197       | 7.56                      | 0.31                                | 28.5                  | 1                          |          |        |                    |                                  |                               |

* $n$-alkanes below detection limit precluded the calculation of CPI, $P_{aq}$, and ACL.
* Key iDGTs below detection limit precluded the calculation of SST from $TEX_{86}^{L}$ and/or mean summer lake water temperature.
* No pigments detected.
* Sterols below detection limit.

Key: terrestrial OM; aquatic OM; marine OM; mixed origin of OM; grass; forest.

Water temperature: warm water, cold water.

1: CPI: carbon preference index (Supplementary Information, Table S4).
2: $P_{aq}$: proxy for aquatic macrophytes (Table S4).
3: ACL: average chain length (Table S4).
4: BIT: branched and isoprenoid tetraether index (Table S4).
5: $TEX_{86}^{L}$: tetraether index of 86 carbon atoms (low) (Table S4).
6: Mean summer lake water temperature (Table S4).

Note: absolute values are probably not appropriate, but the general trend is likely to be indicative of palaeoenvironmental change.

n.d.: not determined because below the detection limit.
The marine transgression (102–81 cm)

At 92 cm core depth (94–92 cm, 5939–5753 14C a BP; Bates et al., 2016) signatures of n-alkanes were absent/below the detection limit. An increasing dominance of aquatic OM (algae n-alkanes) at the expense of terrestrial OM reflects the continuation of the marine transgression (Fig. 6a–c). A much reduced population of anaerobic photosynthetic organisms probably reflects disruption of the chemocline (Fig. 6d).

At 85 cm (84–82 cm, 5862–5612 14C a BP; Bates et al., 2016), a low CPI value of 4.39 (Fig. 5b; Table 3) was recorded. This can indicate a dilution in the terrestrial OM by algal n-alkanes (Fig. 5b; Table 3) even though concentrations are low, as shown by the profile (Fig. 6a; Clark and Blumer, 1967; Ortiz et al., 2004). The presence of algal OM is also confirmed by short alkanoic acids and pigments. The low PnA proxy indicates enhanced terrestrial OM input and a decrease in freshwater macrophytes compared with the samples in earlier horizons (Fig. 5c; Table 3; Ficken et al., 2000).

While microfossil evidence indicates brackish conditions being established from 102 cm, the detection of crenarchaeol at 85 cm depth indicates the onset of production by a small population of marine Thaumarchaeota (Figs 4 and 5a; Table 3). The contrast with other lipid analyses that suggest that the freshwater lake was disrupted from as early as 118 cm could reflect a lag in the production of Thaumarchaeota with them only becoming prominent once stable brackish conditions became established towards the end of the transgression. The absence of bchl’s at 85 cm reveals the disappearance of the chemocline with establishment of a fully oxic water column (Fig. 6d). Such a shift in the primary producer community can result from turbulence in the water column (Squier et al., 2002). It is evident that the sample at 85 cm represents a switch between a turbulent environment (from the pigments) and a stable environment (from the GDGTs) representing the ongoing transgressive event.

The established brackish loch (80–0 cm)

Established marine production with terrestrial OM input is indicated by the presence of crenarchaeol and soil brGDGTs (77–14 cm; Fig. 4). The range of BIT values observed is comparable to open coastal environments (Fig. 5a; Table 3; Hopmans et al., 2004; Doğru Selver et al., 2012). The n-alkanol and n-alkanoic acid distributions and concentrations indicate a dominance of terrestrial over aquatic OM (Fig. 6b).

The similarity of the lipid concentrations to those preserved in the freshwater lake environment is consistent with cessation of the disruption associated with the transgression (Figs 5 and 6). The high CPI values throughout the brackish loch section confirm the predominance of terrestrial OM and lower contribution from algal OM (Fig. 5b; Table 3). The absence of chl pigments in this section of the core is consistent with strongly oxidative conditions leading to total destruction of pigments (Fig. 6d; Hendry et al., 1987).

Signatures of terrestrial vegetation as markers of changing environment

The higher plant-derived n-alkanes show a shift in chain length with depth, the maximum changing from n-C27 (197–155 cm) to n-C31 (from 85 cm; Fig. 6a), reflected in the lower ACL values between 197 and 155 cm and higher values above 85 cm (Fig. 5d; Table 3; Poynter and Eglinton, 1990). Such a shift in the predominance of n-alkane chain lengths has been suggested to reflect a change in the surrounding vegetation from mainly forest vegetation (dominant n-C27) to mainly grasses (dominant n-C31; Cranwell, 1973; Ortiz et al., 2004). This would imply that the Stenness catchment area experienced gradual deforestation from the freshwater period (before ~5939–5753 BP) with grassland dominating after inundation (~5862–5612 BP). This observation provides a tantalizing view of the changing environment and should be a stimulus for further work that could involve identification of more specific grass/forest biomarkers, palynological studies and modelling to gain further insights into the palaeoclimatic conditions (Fisher et al., 2003; Zech et al., 2013; Li et al., 2018).

Palynological analysis of a core from Crudale Meadow, located ~5 km north-west of the Loch of Stenness, suggests a predominance of arboreal pollen (AP) mainly Betula and Pinus sylvestris with <15% herbs, between 8.1 and 5.7 ka BP (Bunting, 1994). A decline in AP to 40% and increase in herbs and Filicales (ferns) are apparent from 5.7 to 4.7 ka BP and today (Bunting, 1994). Similar results were obtained from Blows Moss, ~40 km south of the Loch of Stenness site (Farrell et al., 2014).

The vegetation change is thought to have resulted from a cooling climate and human activity in the surrounding area (Keatinge and Dickson, 1979; Bunting, 1994; Farrell et al., 2014; Bates et al., 2016). The decrease in AP at Crudale Meadow by 5.7–4.7 ka BP coincides with the end of the Loch of
The alternative was applied to the brackish samples (77 °C in March to 13.2 °C in August, giving an average yearly temperature of 10.1 °C; Dalton, 2008). SST estimates could not be inferred from the TEX86 proxy. Mean summer air temperatures estimated from analysis of chironomids from Scotland were consistent with declining summer insolation during the Mid–Late Holocene (1.5–2 °C; Dalton et al., 2005). Structural variations in brGDGTs have been correlated with soil pH, via the CBT index (Weijers et al., 2007). The applicability to lake sediments was established via a modification of the calibration (Tierney et al., 2010; Table S4). The CBT-calculated pH values show a narrow range of mildly alkaline sediments between 7.34 and 8.02 ± 0.66 (Fig. 5e), values consistent with the geology of the area (Kellock, 1969).

**Contextualizing the transgression**

North-eastern Scotland experienced a positive isostatic rebound of +0.53 mm a⁻¹ over the last 6000 years BP (Shennan, 1989) and Orkney experienced a rebound of <0.0 mm a⁻¹ over the last 4000 years BP (Shennan and Horton, 2002; de la Vega-Leinert et al., 2007). The transgression at the Loch of Stenness cannot be attributed to melting of the Orkney island ice sheet, which occurred much earlier, between 16.0 and 14.8 ka (Ballantyne, 2010). By contrast, modelling shows that the Antarctic Ice Sheet continued to thin until at least 6 ka (Mauz et al., 2015), and in some cases until 3 ka (Hein et al., 2016; Small et al., 2019), and has been invoked to explain the Mid–Late Holocene sea-level increase in sites across the globe (Mauz et al., 2015). Notably, however, during the Mid–Late Holocene, sea-level fluctuations at Scottish sites have shown
both transgressions and regressions, indicating that regional factors, such as crustal movements and tidal dynamics (Smith et al., 2019) cannot be excluded. Indeed, local factors such as the natural creation and destruction/modification of bay mouth barriers (lying – 5 m OD in the Loch of Stenness) are likely to have played an important role in the timing and nature of transgressions in the Loch of Stenness (Bates et al., 2016). Shennan and Horton (2002) suggested that Orkney sea levels reached present values 5–4 ka, in agreement with the radiocarbon dates for this core (Bates et al., 2016) and the present study on the Loch of Stenness.

**Conclusions**

The analysis of microfossils from the Loch of Stenness (Orkney) core reveals initial freshwater conditions (200–104 cm depth), followed by a transgression (102–81 cm) leading to brackish conditions (80–0 cm; Bates et al., 2016). The molecular fossil record indicates greater complexity during the transgression than had been apparent from palaeoecological records alone. The initial freshwater lake was a productive environment with input both from in situ aquatic production and from terrestrial runoff (197–155 cm). The first indication of a disruption of the OM balance was recorded at 118 cm, where terrestrial OM decreased and algal OM increased in concentration, possibly indicating an earlier initiation of the transgression than is evident from microfossil analysis (Bates et al., 2016). An increase in aquatic OM production was observed at 92 cm with the marine marker crenarchaeol being detected above 85 cm, consistent with the establishment of stable marine conditions (Fig. 7). In the brackish zone (77–14 cm) marine OM was detected and the extent of terrestrial runoff was similar to that of the freshwater stage, indicating the end of the transgression.

The change in n-alkane chain lengths and ACL values suggest a shift in vegetation from forest to grasses towards the top of the core, supported by pollen data, probably in response to cooling conditions associated with reduced summer insolation as well as human activity. The proxy-calculated SST suggests a cooling trend towards values close to present-day water temperatures at 77, 38, and 14 cm (13.1–10.5 °C, ± 4 °C).

Piloted on a well-constrained Holocene transgression, this study has shown that molecular fossils can add valuable environmental evidence to the information garnered from more conventional estimates of palaeoclimate change from microfossil, palynological and lithological studies. The nature and specific origins of molecular fossils enable a more insightful and refined study of the impact of the transgression on the primary producers inhabiting the basin and the surrounding environment. The widespread nature, greater frequency of occurrence and excellent preservation potential of molecular fossils broadens the scope of palaeoenvironmental studies and unlocks a far wider set of sediments that can be used in the study of sea-level change across the world, including sediments where macro- and microfossils are lacking.

**Supporting information**

Additional supporting information may be found in the online version of this article at the publisher’s web-site.

**Table S1.** Lithological and microfossil analysis from Loch of Stenness (Orkney, Scotland) core 2014-1.

**Table S2.** Radiocarbon data from the Loch of Stenness (adapted from Bates et al., 2016).

**Table S3.** Extended methods for the extraction and analysis of molecular fossils employed in this study.

**Table S4.** Equations for n-alkane-based proxies CPI, ACL and P<sub>ape</sub> GDGT-based proxies BIT, TEX<sub>868</sub>, TEX<sub>898</sub>, mean summer lake water temperature and CBT. Structures of GDGTs discussed in the table are included.

**Figure S1.** Concentrations of chlorophyll pigments in the Loch of Stenness core 2014-1: chlorophyllone (chlorone), phaseophytin a (phe a), pyrophosphate a (pphe a), pyrophosphate b (pphob a), hydroxyphaseophytin a (phe a), purpurin-7-trimethyl ester, chlorin e6, phaseophytin b (phe b), bacteriochlorophyll a (bchl a), bacteriopheophytin a (bph a), pyrobacteriochlorophytin a (bph a), and bacterioviridin a (bvir a).

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**Data availability statement**

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

**Abbreviations.** ACL, average chain length; AP, arboreal pollen; bchl, bacteriochlorophyll; BIT, branched and isoprenoid tetraether; brGDGT, branched glycerol dialkyl glycerol tetraether; bvir a, bacterioviridin a; CBT, cyclization of branched tetraethers; chl, chlorophyll; CPI, carbon preference index; GC-FID, gas chromatography-flame ionization detection; GC-MS, gas chromatography-mass spectrometry; GDGT, glycerol dialkyl glycerol tetraether; iGDGT, isoprenoid GDGT; MAAT, mean annual air temperature; OM, organic matter; SST, sea-surface temperature; UHPLC-DAD, ultra-high performance liquid chromatography with diode array detector.

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