**[Supplementary material]**

**New integrated molecular approaches for investigating lake settlements in north-western Europe**

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**OSM 1: determining a site chronology and sediment geochemistry**

Dating has been mostly provided by AMS radiocarbon dates determined at the University of Oxford Radiocarbon Laboratory. OxCal version 4.4 (Bronk Ramsey 2008) was used for calibrating radiocarbon dates, using the IntCal20 curve (Reimer *et al.* 2020) and for creating age-depth models. *Seda*DNA and faecal biomarkers were extracted from a parallel core taken within 2m of the dated core adjacent to the crannog. Both cores analysed for sedXRF and were correlated using Ti and Fe element data. Clear peaks in Ti and Fe available in both cores were used as tie points which were then used in software package QAnalySeries (Kotov & Pälike 2018) to correlate these cores. This information was then used to transpose the age model of the dated core onto the correlated depths of the core used for *seda*DNA/biomarkers. For geochemistry, core XRF scanning was undertaken using an ITRAX XRF scanner (Croudace *et al.* 2006) directly on the split cores scanned at 2mm resolution using 30kV, 30mA settings and a 15 second count time at the British Ocean Sediment Core Research Facility (BOSCORF) at the National Oceanography Centre, Southampton (NOCS). In total, 39 elements were identified including lithogenic indicators (Si, Al, K, Ti, Zr, Rb) and anthropogenic and heavy metal indicators (P, Ca, Cr, Zn, Ba, Sr, Pb). The resultant elemental intensities, measured in counts per second (cps), were vetted to remove unreliable results which occurred at the boundaries of samples and in particularly coarse sediment horizons. Following data control analysis, the remaining elemental relationships were plotted as ratios to Ti as the conservative detrital element.

**OSM 2: automated SEM-EDS**

Automated SEM-EDS (Pirrie & Rollinson 2011) analysis was carried out using QEMSCAN technology. QEMSCAN is an automated mineralogical analysis system based on a scanning electron microscope that provides rapid determination and quantification of the mineralogy, chemical composition, grain size and shape of a variety of sample types. Data collection is operator independent, with the acquisition of very large data sets, hence the results are statistically reliable and provide highly reproducible mineralogical analyses. The technology has been widely used in the analysis of soil and sediment samples (Pirrie *et al.* 2014). There are various modes in which the QEMSCAN can be run; including particle mineral analysis (PMA) mode where individual particles are mineralogically mapped, bulk mineral analysis (BMA) mode where the bulk mineral assemblage of a sample is very rapidly determined, field image mode where the entire sample is mineralogically mapped and trace mineral search (TMS) mode where only particles with a specified backscatter electron threshold are mapped. In this study the samples were analysed using the PMA measurement mode using a 1.5µm beam stepping interval with ~50 000 mineral grains measured. The raw data acquired during QEMSCAN analysis are based upon classification of the individual X-ray spectra by using a look-up table containing in excess of 600 known mineral phases and chemical compositions. Spectra that cannot be matched to known phases at this point are classified as “others” and the coordinates stored (they can be assigned to a chemical phase at a later date). The raw data are then processed by assigning similar pixel types to single categories which may be a mineral phase, chemical composition, or any other category. In this way, edge and boundary effects can be accounted for and any other phases present with a discrete chemical composition can be assigned to a distinct phase category.

**OSM 3: sedaDNA metabarcoding**

DNA was extracted from lake sediment samples and three negative controls at the ancient DNA (aDNA) dedicated laboratories at the Laboratoire d’ECologie Alpine, University Grenoble Alpes (LECA). For each sample, we extracted DNA from ~15g of wet sediment using the Macherey-Nagel Kit (Düren, Germany), following the manufacturer’s instructions. All PCRs were performed in an aDNA dedicated room, using the g and h universal plant primers for the short and variable P6 loop region of the chloroplast trnL (UAA) intron and including a unique 8 bp long flanking sequence (tag) at the 5′ end to allow parallel sequencing of multiple samples (Alsos *et al.* 2018). For mammal DNA amplification, the MamP007F mammal primer was used for a 60–84-bp fragment of the mitochondrial 16S gene (Alsos *et al.* 2018). DNA amplifications were carried out in 50μL final volumes containing 5μL of DNA sample, 2U of AmpliTaq Gold® DNA Polymerase (Life Technologies, Carlsbad, CA, USA), 15mM Tris-HCl, 50mM KCl, 2.5mM MgCl2, 0.2mM each dNTP, 0.2μM each primer and 8μg Bovine Serum Albumin. Three PCR negative controls were also carried out. All PCR samples (DNA and controls) were randomly placed on PCR plates. Following the enzyme activation step (10 min. at 95°C), PCR mixtures underwent 45 cycles of 30s at 95°C, 30s at 50°C and one min. at 72°C, plus a final elongation step (seven mins at 72°C). Eight individually tagged PCR repeats were made for each sample to increase the chance of detecting taxa represented by low quantities of DNA, as well as to increase confidence in the taxa identified. Equal volumes of PCR products were mixed (15μL of each), and 10 aliquots of 100μL of the resulting mix were then purified using MinElute Purification kit (Qiagen GmbH, Hilden, Germany). Purified products were then pooled together before sequencing; 2 × 100 + 7 paired-end sequencing was performed on an Illumina HiSeq 2500 platform using TruSeq SBS Kit v3 (FASTERIS SA, Switzerland).

Sequence data were analysed using the OBITools software package (Boyer *et al.* 2016). First, direct and corresponding reverse reads were assembled using *illuminepairedend*, and sequences having a low alignment quality score (threshold set at 40) were filtered out. The retained reads were assigned to relevant samples using ngsfilter, keeping sequences matching 100 per cent with tags and allowing a maximum of three mismatches with primers. Strictly identical sequences were then merged (dereplication) using *obiuniq*, keeping information on their distribution among samples. All sequences with only a single copy and/or shorter than 12 bp were filtered out using *obigrep*. *Obiclean* was then used to identify amplification and sequencing errors, using a threshold ratio of 5 per cent for reclassifying ‘internal’ sequences to their relative ‘head’ sequence (De Barba *et al.* 2014). Finally, using the sequences were compared with a the global EMBL database (release r117 from October 2013) by running *ecopcr.* Sequences assigned to non-native taxa were blasted to check for potential wrong assignments (http://www.ncbi.nlm.nih.gov/blast/).

Extreme caution must be taken before accepting a taxonomic assignment in an environmental sample (Taberlet, *et al.* 2007; Ficetola *et al.* 2015; Lahoz-Monfort *et al.* 2016). To reduce risk of misidentifications, only sequences matching 98 per cent to reference library entries and occurring as at least 10 reads per PCR repeat for plants and five reads per PCR repeat for mammals were kept. The following were also removed: (1) sequences having higher frequencies in negative controls than in samples; (2) sequences occurring in <3 repeats in total (i.e. across all samples); (3) sequences belonging to exotic food plants and thus suspected to be contaminants; and (4) sequences suspected to be droplet contaminants or overflow from samples from another study run at the same time. One complete sample, which appeared as an outlier in terms of low number of reads and repeats, was excluded. By applying these thresholds, rare taxa were possibly missed but potential errors were removed.

**OSM 4: lipid biomarkers—faecal stanols and bile acids**

Faecal lipid biomarker analysis was conducted following standard protocols based on Bull *et al.* (2002) and outlined in Mackay *et al.* (2020). Briefly, 10μL of androstanol (0.1mg mL-1) and 10μL of hyocholic acid (0.1mg mL-1) were added as internal standards to each sample of approximately 1g of dried, homogenised sediment. Total lipids were extracted with solvents (DCM:MeOH, 2:1, *v/v*) using microwave assisted extraction (heated to 70°C for10 mins, then held at 70°C for 10 mins; saponified using 5M sodium hydroxide in MeOH and separated into neutral and acid fractions using aminopropyl SPE columns. The acid fraction was methylated using 3mL of trimethylsilyldiazomethane (TMS-DAM) in toluene/methanol (4:1 *v/v*). Silica gel column chromatography was used to isolate the hydroxylated carboxylic acid fraction (containing the bile acids) from the methylated acids. Methylation of the acid fraction was achieved using 3mL of trimethylsilyldiazomethane (TMS-DAM) in toluene/methanol (4:1 *v/v*). The sterol and bile acid fractions were derivatised using 30μL of BSTFA+TMCS (99:1 *v/v*) and dissolved in 50–100μL of ethyl acetate prior to gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analysis. GC-MS analyses were performed on an Agilent 7890B GC injector (280°C) linked to an Agilent 5977B MSD (electron voltage 70eV, source temperature 230°C, quad temperature 150°C multiplier voltage 1200V, interface temperature 310°C) in full scan mode (50–600 amu/sec). Separation was performed on an Agilent fused silica capillary column (HP-5, 60m × 0.25mm ID × 0.25um df), with Helium as a carrier gas. The sample (1μL) was injected in splitless mode (one min. splitless time). Sterol derivatives were analysed using the following temperature programme: 50°C (held for two mins) to 200°C at 10°C min−1 then to 300°C at 4°C min−1 and held for 20 mins. Bile acid derivatives were analysed using the following temperature programme: 40°C (held for one min.) to 230°C at 20°C min-1 then to 300°C at 2°C min-1 and held for 20 mins. GC-MS peaks were identified through comparisons with known mass spectra (NIST08) and standards where possible. Analytes were quantified based on internal standards. The dominant faecal matter source was identified using the ratio of deoxycholic acid (DCA) to lithocholic acid (LCA) ratio (Prost *et al.* 2017). Based on modern experimental data, the values of this ratio can be ascribed in the following way: <0.4 pigs and/or geese; 0.6–4.5 humans and/or horses; >5 ruminants (cattle, sheep and goats). Whilst the dominant faecal source can be identified using these ratios, this does not preclude the presence of other faecal sources in smaller quantities.

**OSM 5: stable isotopes (δ13C and δ15N)**

For δ13C, dried sediments are placed in 5% HCl at room temperature overnight with occasional stirring to remove carbonates before δ13C analysis. Sediments are then washed three times using de-ionised water over quartz-micro filter paper (Whatman QM-A) using a vacuum filtration system, dried at 40°C and subsamples were weighed into tin capsules. For δ15N, untreated subsamples were weighed into tin capsules to provide ~ 0.1mg of nitrogen based on the TN content of the sediment. Samples were analysed by combustion using a ThermoFinnigan FlashEA 112 elemental analyser coupled on-line via a Conflo III interface to a Deltaplus XL isotope ratio mass spectrometer at the British Geological Survey (Keyworth, UK). Stable isotopes are reported in delta notation versus VPDB for δ13C and versus AIR (atmospheric N2) for δ15N by comparison with the laboratory standards BROC2, which has been calibrated against the international reference materials IAEA-N-1 and IAEA-N-2. Analytical precision based on repeat measurements of the laboratory standards <0.04‰ for δ13C and <0.2‰ for δ15N (1σ).

**OSM 6: biogenic silica (BSi)**

Biogenic silica (BSi) concentrations were determined using a modified version of the DeMaster (1979) wet-alkaline digestion and timed sequential extraction procedure. Round-bottomed polyethylene tubes containing 38ml of 0.5M sodium hydroxide (NaOH) solution were placed in a water bath and heated to 85°C. Each batch contained 40 sediment samples, two blanks and four replicates to estimate the method reproducibility. 40mg of freeze-dried, ground sediments were added to the sample tubes, which were shaken to ensure that all sediment particles were exposed to the NaOH solution. Dissolution of BSi was complete after 60 minutes, when a 1ml aliquot of the NaOH solution was extracted. Three further aliquots were extracted at 90, 120 and 200 minutes. The aliquots were dyed using molybdate blue solution and reduced for spectrophotometric measurement at 812nm alongside sodium fluorosilicate standard solutions. A least-squares linear regression through the absorbance values of the timed-aliquots corrected for mineral dissolution during the digestion and the amount of BSi was determined using the intercept of this line. Absorbance values were converted to per cent BSi using the standard calibration curve and the sample weights and flux values were calculated to account for sedimentation rates.

**OSM 7: crannogs—monuments at risk?**

The South-west Scotland Crannogs Condition Survey (Henderson *et al.* 2003; Henderson 2004) documented the erosion and decay of crannogs in this area. The vulnerability of crannogs to wave erosion is clearly influenced by the size, shape and orientation of lakes with erosion commonly on the outer parts of the crannog facing the fetch and siltation on the leeside generally between the crannog and shore (Henderson *et al.* 2006). Those exposed to the greatest fetch will be most vulnerable to wave-erosion and lee-side siltation, as is the case with five of the 15 crannogs in Table S1. Using estimates from the original revetment of the crannogs, or early surveys, some very approximate calculations of the average long-term rates of loss can be made. In the case of Lough Yoan, the southern crannog is less than half its original size (FCM 1977 057 and the star-like shape) and the Northern crannog has also been severely reduced in size based upon the location of perimeter piles. The crannog at Barhapple, south-west Scotland (Table S1), was surveyed in 1884 and had entirely gone by 2000 giving a minimum average loss rate of 0.8 per cent per year. This loss rate is similar, if a bit higher, than the rates calculated by Henderson *et al.* (2006) for Ballydoolough, Co. Galway, Ireland (0.46 per cent yr-1), Barlockhart (0.4 per cent yr-1) and Dormans Island (0.5 per cent yr-1), both in Dumfries & Galloway, Scotland. Although at these rates of erosion suggest it will take over 100 years for the crannogs to be eroded away entirely—it is extremely unlikely that the rate of loss is linear or spatially uniform due to the greater exposure and perimeter relative to area as the size of the island decreases.One of the reasons that the number of crannogs recorded is almost certainly an underestimate, is that many crannogs have already been eroded to below the water-level, and have become invisible (e.g. Barhapple Loch, south-west Scotland, Clonmin crannog, Northern Ireland, in Table 1). This can be partially rectified by underwater survey (Dixon 1991), and indeed this can result in spectacular finds (Garrow & Sturt 2019), but this is not possible for all lakes, and there comes a point when there is little if any bathymetric evidence remaining. If submerged sites are found then the techniques illustrated here can be used in addition to, or in place of, diving and underwater excavation. Given this coring may provide a cost-effective way of salvaging as much information as possible without excavation, it is suggested that at the regional level, crannogs at risk should be subject to assessment and either, or both, proximal sediment coring and through crannog coring and with at least some of the techniques outlined here.

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**Table S1. Metadata for sites cored just prior to and under the *Celtic Crannogs and Connections Project*.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Lake/Loch/Lough****Location**  | **Lake size in****km2 (water depths m)** | **Name/note & date of coring** | **Crannog period** | **Finds** | **Proxies**  | **Condition and risks** | **References** |
| Cults, SW Scotland54.9036 -4.9321 | 0.06 (1–3) | Cults 1, Island crannog(2012) | LIA 153–121 BC | Timbers, glass bead, finely carved ard, wooden vessel, stave-like objects | XRF, LOI, diatoms, pollen | Moderate to severe, fluctuating water levels  | Cavers & Crone 2017; Fonville *et al.* 2017 |
| (1–7.65) | Cults 3, promontory crannog(2012) | LIA *c*. 730–210 BC | Timbers, planking, structures, hearths, shale or cannel coal bracelet, whetstones | Pollen, diatoms | Moderate to severe, fluctuating water levels |
| Barhapple, SW Scotland54.8965 -4.7150 | 0.06 (0.7) | Unnamed with a causeway (2015) | EIA2130±50 BP | Shale ring, wooden beams, paddle, logboat  | XRF, LOI, diatoms, pollen | Eroded compl., submerged (1884 survey, min. est. 17 m2 yr loss, 0.84% per yr.) | Canmore 62139Wilson 1882; Henderson *et al.* 2003, 2006 |
| White Loch of Myrton. SW Scotland54.757545 -4.552335 | 0.2 | Unnamed | LIA 715–206 BC | Furze, faggots, fern, brushwood, Slabs, charcoal, daub, whetstone, barley, wheat  | XRF, P, sedaDNA, (exc. 2016) | Minor erosion & decay, leeside siltation, root penetration | Canmore 62814Cavers & Henderson 2002 |
| Derryhowlaght East,Fermanagh, NI54.275472 -7.540083 | (2.5) | Unnamed (2015) | Early med AD 666–872 | Timbers, wattle panels, animal bone (horse, pig), rotary quern, iron slag | XRF, LOI, diatoms | Eroded or windward (N), silted on lee | Williams 1993; Fonville 2015 |
| Ross Lough, Fermanagh, NI 54.3685 -7.7910 | 0.8 (2) | 2-3 unnamed crannogs, eastern one attached to land by a causway, throughflow lake | IA? | Bronze ring, bronze spiral, pick(stone), flint blade, also animal bones, human bone, artefacts (from nearby Sillees R) | XRF, diatoms | Minor erosion, siltation by causeway | Carroll 1992; Fonville 2015 |
| Roughan, Co Tyrone, NI54.559687 -6.721276 |  | One crannog in lake centre used historically as a refuge | Med | Three bronze spears, quern with Celtic cross dec., sherds, bone, hist documented 1601 | Strat, XRF,  | Slightly eroded | Pringle 1935 |
| Aughlish, Co Tyrone, NI 54.504440 -6.853009 | 0.06 (1) | Unnamed but associated with the O’ Donnellys, lough largely silted up | BA/IA, Med/E. mod | Two canoes, pottery animal bone (pig, cattle),  | XRF, LOI | Comp. silted, disturbed | Carver & Donnelly 2011; Thurston & Plunkett 2012; Gray 2015; Murray 2016 |
| Barry, Fermanagh, NI 54.272785 -7.585053 | 0.12 | Aughey crannog | EM cal. AD 722–976 | – | XRF, ADM | Erosion & siltation (N-E), root penetration,  | FER 230:082 |
| Catherine, Fermanagh, NI 54.700834 -7.434980 | 0.29 | Island McHugh (crannog phase) | EMAD 601–*c*. 1000 | Timber bld., animal bone (pig, sheep, cattle), barley, oats, elder, bone comb, glass bead | XRF, | Disturbance, minor erosion | Murray 2016 |
| Yoan, Fermanagh, NI Lat:54o19’49.25Long: 7o36’49.31 | 0.48 | NorthernGortgonnell/Killyherlin | EMAD 1044–1264 (pile);AD 777–994; AD 756–962 (sed.) | Whetstones, quernstones animal bone (two incisor teeth, several fragments of bone, two fragments of ribs and part of a jaw-bone) | XRF, P,D, Ch, Cl, LB, sedaDNA, QS,  | Highly eroded | Wood-Martin 1886 (FCM\_1978\_133) |
| Yoan, Fermanagh, NI Lat: 54o19’38.21Long: 7o36’29.46 | SouthernDrumcrin | EMAD 714–1103 | Fragment of rotary quern MUC ×3: decorated rim sherd, plain body sherd, base sherd (DoE), wood chips | XRF, pollen, spores | Highly eroded, only an irregular form remaining | (FCM\_1977\_057) |
| Finlaggan, Islay55.834398 -6.1734381/ 55.828258 -6.174297(2019) | 0.63 | ‘Bishops’ Eilean Mhuireill crannog | EM | Crucifix-cross, quern fragment | Pm, XRF, LB, sedaDNA | Very minor erosion | SM5789, D. Caldwell *pers. comm*. |
| ‘Council Isl’ Eilean na Comhairle crannog | EM89 BC–AD 598 | Exc. (underwater, bone, sherds) |  | Moderate erosion of fetch side (N) |
| Clonmin, Fermanagh, NI 54.193355 -7.429141(2019) | 0.10 | Unrecorded  | ? | None | Pm, XRF, LB, sedaDNA | Comp. Eroded, subm. | − |