Journal of Archaeological Science 59 (2015) 159–168

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

# Apatite for destruction: investigating bone degradation due to high acidity at Star Carr

K. High [a, *], N. Milner [b], I. Panter [c], K.E.H. Penkman [a]

[a] Department of Chemistry, University of York, YO10 5DD, UK
[b] Department of Archaeology, The King's Manor, University of York, YO1 7EP, UK
[c] York Archaeological Trust, 47 Aldwark, York YO1 7BX, UK

ARTICLE INFO

*Article history:*
Received 17 June 2014
Received in revised form
28 March 2015
Accepted 3 April 2015
Available online 20 April 2015

*Keywords:*
Star Carr
Bone
Acidity
Diagenesis
SEM
Amino acid racemization
Powder X-ray diffraction

ABSTRACT

In order to more fully understand the effects of high acidity on bone at the Mesolithic site of Star Carr, a suite of analytical techniques has been used to assess the effects on the inorganic and organic fractions of treating a range of bone types in different strength sulfuric acid solutions. These include mass loss analysis, scanning electron microscopy, chiral amino acid analysis, and powder X-ray diffraction. Loss of bone mineral is shown to be severely accelerated at low pH and this ultimately leads to increased breakdown of the bone collagen. Archaeological samples are significantly more at risk than modern samples. Reaching an understanding of the effects of increased acidity on organic artefacts through studies such as this has important applications in determining the future management not only of Star Carr, but other sites with similar chemical environments.



## 1. Introduction

Star Carr is a Mesolithic hunter-gatherer site located in the Vale of Pickering, North Yorkshire, renowned for the discovery of a large number of extremely well preserved organic artefacts rarely found in sites of this age (Milner et al., 2011). Excavations between 1949 and 1951 (Clark, 1954) and in 1985 and 1989 (Mellars and Dark, 1998) uncovered remarkable organic finds, including over 20 rare antler frontlets, thought to be headdresses, and an unprecedented number of barbed points (over 190), carved from red deer antler and used for hunting. These have provided archaeologists with a wealth of information regarding life in Britain during this time.

However, the discovery of severely degraded bone, antler and wood in recent excavations (2006–2014) has indicated that the site conditions may be no longer conducive to the levels of preservation seen in the original excavations. In particular, the discovery of heavily demineralised bones, termed 'jellybones', raised concerns for any bone as yet uncovered (Milner et al., 2011). In 2009, soil pH values <2.5 were recorded at the site, hypothesised to be due to sulfuric acid formation (Boreham et al., 2011). As previous studies show that high acidity has a detrimental effect on the survival of bone (e.g. Gordon and Buikstra, 1981; Nicholson, 1996), it has been hypothesised that this unusually high acidity is the key factor facilitating this decline in organic preservation. Instances of similar pH levels in archaeological deposits are known: examples include Yoxall Bridge, where a soil pH of 2 was thought to be caused by underlying sulfur-rich mineral deposits (Brown et al., 2010); and areas of the Bronze Age site of Flag Fen, where a pH of approximately 3.5 has been reported (Powell et al., 2001). However, studies at these sites have not investigated the preservation potential for bone in such acidic soils. Although other studies have taken an experimental approach to modelling bone diagenesis (e.g. Turner-Walker and Peacock, 2008; Karr and Outram, 2012), research into the effects of the pH of the burial environment has been limited to environments with only mildly acidic sediment (e.g. >pH 3.2, Nicholson, 1996). This study therefore aims to test how destructive the high acidities found at the Star Carr site are to bone, and in particular to the stability of its protein fraction, controlling for other site conditions.

To this end, laboratory-based degradation experiments under acidic conditions were performed using a range of modern and

* Corresponding author.
*E-mail address:* Kirsty.high@york.ac.uk (K. High).

http://dx.doi.org/10.1016/j.jas.2015.04.001
0305-4403/

archaeological bone. A major aspect of this study is the assessment of the effectiveness of various analytical methods for detecting low levels of bone deterioration. The techniques have been selected due to their routine availability, relative inexpensiveness and ability to provide complementary information on the inorganic and organic fractions. They span those useful for assessing bulk (macroscopic and microscopic) degradation, such as mass loss analysis and scanning electron microscopy (SEM), alongside the analysis of molecular degradation (powder X-ray diffraction (p-XRD) and amino acid analysis).

Identical experiments were carried out on wood samples and are reported elsewhere (High et al., in press).

## 2. Materials and methods

### 2.1. Materials

Modern long bone and rib samples were obtained from a butcher and identified by the butcher as sheep, with long bone A being from a more mature animal than long bone B. A large mammal rib bone was obtained from Star Carr during excavations in 2010. Modern samples were de-fleshed and all bone samples sliced into 3 mm cross-sections using a diamond edged water cooled band saw.

### 2.2. Method

Solutions of differing concentrations of sulfuric acid were made in order to mimic the low pH groundwater evident at the Star Carr site. Although the behaviour of acids in the burial environment is likely to be more complex, using only sulfuric acid enabled control over the levels of acidity, and allows changes in pH to be more easily monitored. Stock solutions of sulfuric acid at pH values of 1, 2, 3 and 5 were made using MilliQ water and 12 M sulfuric acid (Fisher Scientific). The accurate pH of each stock solution was recorded using a calibrated glass pH probe (Denver instrument) and kept to within 0.1 pH units of the selected pH throughout the experiment. Each sample was placed in a sterile glass screw-top vial and filled with 50 mL/g of the relevant solution. Two hydrological regimes were mimicked: the first to replicate conditions where there is limited movement of solution ('stagnant' conditions; S), and a second where the solution was replenished, ('dynamic' site hydrology; D). Experiments were carried out at room temperature (RT) and at 80 °C in order to accelerate decay under laboratory conditions. The experimental conditions are summarised in Table 1.

Periodically (approximately weekly) sub-samples of the supernatant liquid were taken from each sample and the pH of the remaining solution recorded. At these points, the solution was replaced in the 'D' samples.

### 2.3. Analytical methods employed

#### 2.3.1. Bulk assessment

At each sampling interval, the pH of each solution was recorded using a calibrated glass pH probe (Denver instrument). Due to potential evaporation from samples, this method is not quantifiable, but provides a time dependant qualitative assessment of bone mineral dissolution. All samples were also observed visually and recorded photographically throughout.

At the end of the experiment, the mass loss in each bone sample was calculated as a percentage of the starting mass. As mass loss occurs by dissolution of the sample, this provides a broad measure of sample degradation.

#### 2.3.2. Microscopy (SEM)

A number of microscopic techniques have been shown to be useful for the characterisation of bone deterioration, including thin-section microscopy (e.g. Jans et al., 2002) and transmission electron microscopy (e.g. Koon, 2006). Here, histological analysis of the bone samples has been carried out using scanning electron microscopy (e.g. Turner-Walker and Peacock, 2008). This was selected due to the ease of sample preparation, and the macroscopic analysis obtained determined to be most appropriate as a complementary method to chemical techniques.

A small selection of samples was analysed by scanning electron microscopy (SEM). Minimal sample preparation was carried out following a general method (M. Stark, pers. comm.). A small subsample was dried and mounted onto an aluminium pin-stub and earthed using silver glue. Samples were sputter coated with a 7 nm layer of gold/palladium and images obtained under vacuum using a JEOL JSM-6490LV scanning electron microscope.

#### 2.3.3. Chemical analysis

*2.3.3.1. Powder X-ray diffraction (p-XRD).* Bone mineral, or hydroxyapatite (HA; $Ca_{10}(PO_4)_6(OH)_2$), is naturally low in crystallinity, as its deposition around the collagen matrix is biologically constrained (e.g. Fratzl et al., 2004). It has been proposed that with increasing bone diagenesis in archaeological samples, an increase in crystallinity is seen (e.g. Bonar et al., 1983; Pleshko et al., 1991). This increase in crystallinity can be detected on the molecular level using several different analytical techniques including infrared spectroscopy (e.g. Pleshko et al., 1991), Raman spectroscopy (e.g. Raghavan, 2011) and small angle X-ray scattering (e.g. Hiller and Wess, 2006). Whilst spectroscopic techniques can be affected by

**Table 1**
Summary of time points (in weeks) and experimental conditions for each bone type. Underlined figures indicate where the time point was shorter than intended due to rapid dissolution of the bone.

| pH | T | Time points (weeks) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Long bone A | | Long bone B | | Rib bone | Arch. Rib |
| | | D | S | D | S | D | D |
| 1 | Room temperature | 6,8,16 | 6 | 6 | 6 | 6,16 | 6,16 |
| 2 | | 6,8 | 6 | | | 6 | 6 |
| 3 | | 6,8,16 | 6 | 6 | 6 | 6,16 | 6,16 |
| 5 | | 6,8 | 6 | | | | |
| Water | | 6,8 | 6 | 6 | | 6 | 6 |
| 1 | Heated (80 °C) | 6,<u>4</u> | 6 | <u>3</u> | 6 | <u>2,2</u> | <u>1,2</u> |
| 2 | | 6 | 6 | | | 6 | 6 |
| 3 | | 6,16 | 6 | 6 | 6 | 6,16 | 6,16 |
| 5 | | 6 | 6 | | | | |
| Water | | 6 | 6 | 6 | | 6 | 6 |