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ZooMS: making eggshell visible in the archaeological record

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ABSTRACT

Avian eggshell is a common component of many archaeological deposits, but its archaeological potential remains largely unexplored. The most obvious reasons are two-fold. Firstly, despite its abundance on many sites, eggshell is often overlooked during excavation. Even when it is recovered, small fragmented remains are difficult to identify taxonomically. Here we introduce a minimally destructive qualitative analytical technique for taxonomic identification of eggshell fragments based on highly sensitive mass spectrometry and peptide mass fingerprinting (ZooMS), and illustrate its application to eggshell recovered from the Viking Age urban site at Hungate, York. We adopt a more extreme version of the method of bleach treating used to prepare ancient eggshell for DNA analysis, followed by conventional peptide mass fingerprinting using MALDI-ToF mass spectrometry. The development of this technique will allow future research to make better use of eggshell fragments recovered from archaeological sites.

1. Introduction

1.1. Brief history of egg use

Bird eggs are of high nutritional value, providing consumers with all of the essential amino acids, a range of vitamins and minerals, and important trace elements. They are available at predictable times and locations, often in high densities (Serjeantson, 2009; Sidell, 1993a, 1993b). Although they are a seasonal resource in most areas, they are portable and can remain edible for a number of months after collection (e.g. Kightly, 1984; Serjeantson, 2009). It is therefore not surprising that eggs have formed a significant component of many human diets. The shells themselves have been used as liquid containers and as raw material for jewellery, whilst the contents have often been used as a binding material in paints (Serjeantson, 2009; Texier et al., 2010; Wadley, 1992). Eggs have also held symbolic importance in many cultures (most often as symbols of fertility and/or rebirth) and have been used in various burial practices (Serjeantson, 2009; Sidell, 1993a, 1993b).

Ethnographic accounts of egg collection from north-eastern England in the early 20th century show that exploitation of seabird colonies was highly targeted towards particular species (Kightly, 1984). For example, collectors could expect a greater price for eggs of guillemots and razorbills than for those of other seabirds; they would not take gannet eggs as these were considered too rare; and they would actively destroy gull eggs as they felt that these were out-competing the other species (Kightly, 1984). In these communities, collection was performed almost exclusively by young men, who would work the steep sea cliffs systematically in teams of four (Kightly, 1984). For the Huna Tlingit of the Pacific north-west of North America, the annual collection of gull eggs signals the end of winter, of food shortages and of difficult travel weather, and is considered an exciting time of year, in which children participate actively in egg collection (Hunn et al., 2003). These examples serve to illustrate that a range of approaches to, and attitudes towards, egg collection exists between different cultures in different areas.

Although collection of wild eggs remains important to some remote and/or aboriginal societies, it has largely ceased in many parts of the world (Serjeantson, 2009). The cessation of wild egg collection has been primarily due to the prolific laying capacity of the domestic chicken: these are relatively easy to keep and can provide eggs throughout the year, and therefore provide the vast majority of eggs consumed by modern people. Domestic geese, ducks and turkeys also provide eggs in some parts of the world, while eggs of other species have been considered delicacies in some areas.

However, there are large gaps in knowledge regarding use of wild bird eggs: there are no accounts available for most societies, eggshell fragments may not be recovered from archaeological sites even when they are present, and these are often extremely difficult to identify taxonomically when they are recovered (Section 1.2).

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Although broad patterns of domestication are known for the major modern domestic species (chicken, duck, goose, turkey), there is poor understanding of many aspects (for example, regarding the extent to which people used wild and domestic resources) (Coltherd, 1966; Harrison, 1980; Liu et al., 2006; Manaseryan and Balyan, 2002; Storey et al., 2007, 2010; Tixier-Boichard et al., 2011; Tyrberg, 2002; West and Zhou, 1988). This is unfortunate, as exploitation of wild bird eggs was an important interaction between many cultures and the ecosystems in which they lived. The development of an analytical system which will increase the archaeological potential of eggshell is timely.

1.2. Eggshell in the archaeological record

Eggshell is extremely durable in non-acidic soils, and is a common component of many archaeological sites. Despite the abundance and durability of the material, archaeological applications of it remain surprisingly limited (Section 1.3). This is for two main reasons. There are often difficulties with recovery: eggshell is usually preserved as small fragments (Fig. 1) which are easily overlooked if fine sieving of sediments is not performed during excavation. Furthermore, there is arguably little value in collection as it is very time-consuming to attempt species identification. Despite this, recent reports of the exceptional preservation of DNA in eggshell (Oskam et al., 2010, 2011) highlights the potential value of this resource. We introduce a variant of ZooMS (Zooarchaeology by Mass Spectrometry), designed to rapidly taxonomically identify preserved eggshell, which will allow the archaeological potential of the material to be more fully realised.

1.3. Current archaeological applications of eggshell

The vast majority of current archaeological and geochronological applications of eggshell have utilised large eggshell fragments from the most basal group of birds, the ratites (order Struthioniformes) (Brooks et al., 1990; Hackett et al., 2008; Johnson et al., 1997; Medina et al., 2011; Miller et al., 1992, 1999, 2000; Oskam et al., 2010, 2011; Texier et al., 2010). Ratite eggshell is relatively easy to identify: it is far thicker than the eggshell of other bird orders, and the taxonomic status of a specimen can often be deduced on the basis of geographical location, as many of the suborders of the Struthioniformes are (or were) endemic to particular regions (*but see* Oskam et al., 2011). However, in most parts of the world, ratites are either absent, or humans and ratites were not coeval for long.

Preserved non-ratite eggshell fragments are less often recovered or identified. Recovery of the material typically requires fine sieving of sediments using at least a 5 mm (often smaller) mesh. The most widely used method for identification of these fragments uses scanning electron microscopy to measure a range of internal parameters, which can then be compared with a reference collection (Sidell, 1993a, 1993b). There is a high level of variation in many of these parameters; there can be close similarities, and even overlap, between different taxa (Sidell, 1993a, 1993b). This problem is exacerbated in archaeological specimens, as structural diagenesis can modify the dimensions of the parameters used. This identification technique also requires a relatively large amount of time per sample (Sidell, 1993a, 1993b). Analysing a sample set large enough to be considered representative of the deposits found in archaeological sites, where fragments can number in the hundreds or even thousands (and might theoretically all derive from a very few shells) would be a major undertaking using this technique.

Recently a method has been devised for rapid species identification of bone (ZooMS: Buckley et al., 2009, 2010; Richter et al., 2011; van Doorn et al., 2011) using peptide mass fingerprinting. Here we develop a variant of ZooMS as a robust, reliable, accessible and rapid means of identifying archaeological eggshell fragments. Other recent studies have reported recovery of DNA from preserved eggshell, and utilisation as a taxonomic identifier (e.g. Oskam et al., 2010, 2011). Although DNA analysis is potentially capable of a greater degree of resolution than the system proposed in this paper (e.g. Coghlan et al., 2012), there are several advantages of this technique. It can provide taxonomic information for very small (<1 mg) fragments, allowing analysis of entire eggshell assemblages; current methods for isolation of DNA from eggshell require more material than this (Egloff et al., 2009; Oskam et al., 2010). It does not require preservation of shell membrane or cuticle, both of which are rarely found in archaeological specimens (Lee and Prys-Jones, 2008). It can process entire eggshell assemblages, and has a high success rate. Another major advantage is that, at present, this technique is less costly than DNA analysis. This technique may aid the development of DNA analysis of archaeological eggshell by identifying fragments which are most suitable for subsequent DNA analysis.

1.4. Eggshell structure

Eggshell (Fig. 2) is composed of calcite, the most thermodynamically stable crystalline form of calcium carbonate at surface conditions (Arias et al., 1993; Becking, 1975; Nys et al., 2004). The

Fig. 1. Eggshell fragments from Hungate, York (context 49599).

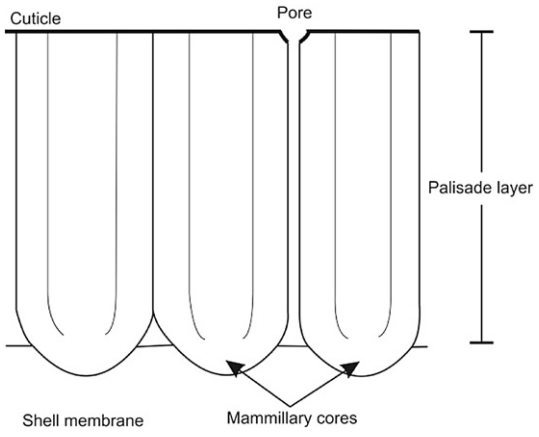

Fig. 2. Generalised schematic of the internal structure of eggshell (adapted from Becking, 1975). No measurements are given as these vary between taxa. The polycrystalline mammillary cores are deposited on the external surface of the shell membrane, and act as seeding sites for the large, vertically oriented calcite crystals of the palisade layer. This layer provides the mechanical strength of the shell, and a network of pores passes through it to allow water and gas exchange. In some species, a thin vertical crystal layer (not shown here) is located between the external surface of the palisade layer and the cuticle. A few species lack a cuticle.

basic structure of the material is highly conserved across all birds, although differences do exist at a more specific level (e.g. eggs of some species lack a cuticle, and instead have a waxy coating) (e.g. Becking, 1975).

1.5. Eggshell proteins: inter- and intra-crystalline networks

A network of proteins is intimately associated with eggshell, and controls the deposition and regulation of the material (Gautron et al., 1997; Hincke et al., 1995, 2010; Lakshminarayanan et al., 2002, 2005, 2006; Mann and Siedler, 2006; Nys et al., 2004). Different C-type lectin proteins have been identified in a range of ratite, galliform (chickens and allies) and anseriform (ducks and allies) species, and are believed to play important roles in eggshell formation (Freeman et al., 2010, 2011; Lakshminarayanan et al., 2002, 2005; Mann and Siedler, 2004, 2006).

When exposed to a powerful oxidant, some proteins within calcium carbonate biominerals are not destroyed. These so-called intra-crystalline proteins have been identified in a range of biominerals (Berman et al., 1988; Crenshaw, 1972; Crisp et al., 2012; Demarchi et al., 2011; Penkman et al., 2008, 2011; Sykes et al., 1995; Towe, 1980; Towe and Thompson, 1972; Walton, 1998), although their precise spatial relationship with the crystalline phase remains unclear. Recent transmission electron microscopy (TEM) of molluscan and algal biominerals reveal spherulitic Fresnel contrasts of a few nanometres, which probably correspond to these intra-crystalline proteins (Okumura et al., 2010, 2012). These proteins are probably not subject to the range of diagenetic influences which may be experienced by those exposed to the external environment (e.g. soil pH, hydrological conditions, microbial attack, infiltration by non-indigenous biomolecules) (e.g. Penkman et al., 2008), and are therefore a viable target for development of a taxonomic identification system for archaeological eggshell.

All of the experiments pertaining to method development and building of the reference collection were conducted on modern and museum eggshell. In order to test whether the extraction procedure and analytical techniques described above are applicable to archaeological material, a pilot study was conducted on the eggshell excavated by York Archaeological Trust (YAT) from the urban site of Hungate, York.

2. Methodology

2.1. Building the reference collection

Eggs of some species (chicken, turkey, duck and ostrich) were obtained commercially. British law prohibits the collection and ownership of wild bird eggs (Wildlife and Countryside Act, 1981). The majority of the other species represented in the collection (56 species in 13 orders – Supplementary information) were drawn from the Victorian and Edwardian egg collections held by Yorkshire Museums Trust (mid-19th–early 20th century). These collections were catalogued, and their suitability for destructive research established by curatorial staff on the basis of a range of criteria (e.g. condition, quality of attached information, rarity, specific research value): the system used closely followed that of Russell et al. (2010). Very small fragments (1–5 mg) were removed from selected eggshells using fine scissors. Some specimens were also provided by J. Sidell (English Heritage) and by J. B. Kristensen (Zoological Museum, University of Copenhagen, Denmark).

2.2. Extraction procedure

Modern and museum samples were washed using ultra-pure water, and the shell membranes (where present) were carefully removed by hand. Archaeological samples were cleaned by sonication in ultra-pure water before any residual dirt was removed by hand. Samples were left to air dry once clean. For archaeological specimens, a small piece was removed from each shell fragment using fine tweezers.

For ZooMS analysis, an intra-crystalline fraction was isolated: this is an established approach in studies of biominerals (e.g. Berman et al., 1990; Collins et al., 1991; Collins and Riley, 2000; Penkman et al., 2008, 2011; Sykes et al., 1995) (Fig. 3). Fragments were weighed into sterile 2 mL Eppendorf tubes, and exposed to strong bleach (sodium hypochlorite, 12% w/v) at a concentration of 50 μL/mg sample for 7 days, in order to oxidise inter-crystalline proteins. The fragments were then thoroughly rinsed in ultra-pure water, briefly suspended in HPLC-grade methanol, air-dried, and the calcite partially dissolved in dilute (0.6 M) hydrochloric acid (HCl) at 4 °C over seven days to release a portion of the intra-crystalline proteins. This approach left a non-dissolved eggshell core to buffer the solution at pH ≈ 7, yet still released a sufficient volume of proteins for mass spectrometry. The lower limit of fragment size to which this technique can be applied is currently unknown: adequate concentrations of proteins for identification by MS have been recovered from fragments originally weighing less than 500 μg, which still leave a substantial core. This means that this technique is never fully destructive; some material remains intact after analysis.

Released proteins were denatured using 0.01 M dithiothreitol (DTT) at a concentration of 1 μL per 2 μL sample at 60 °C for one hour. Cysteine residues were then alkylated using 0.05 M iodoacetamide (IAA) at a concentration of 1 μL per 3.3 μL sample at room temperature in dark conditions for 45 min in order to prevent formation of new disulphide bridges. Following extraction, standard ZooMS protocols were used (van Doorn et al., 2011). Briefly, proteins were digested with 4 μL of 0.4 μg/μL porcine trypsin (Promega, Southampton, UK) in trypsin re-suspension buffer (Promega, Southampton, UK) at 37 °C. Digestion was stopped after 24 h by addition of trifluoroacetic acid (TFA) at a concentration of 0.5–1% of the total solution. Solid phase extraction was performed on BioVyon C18 10 mg 96 well plates (Porvair, Fareham, UK) conditioned (as per manufacturer's instructions) with 50% acetonitrile (ACN) in 0.1% TFA, and equilibrated with 0.1% TFA in ultra-pure water. Samples were then loaded and the unbound fraction washed off in 0.1% TFA in ultra-pure water, before the peptides were eluted in 75 μL of 50% ACN in 0.1% TFA. 1 μL of this eluent was spotted in triplicate on an MTP384 Bruker ground steel MALDI target plate. On each spot, 1 μL of matrix (α-cyano-4-hydroxycinnamic acid; 10 g/L in 50% ACN in 0.1% TFA) was mixed with the sample. Samples were analysed in positive mode on the Bruker

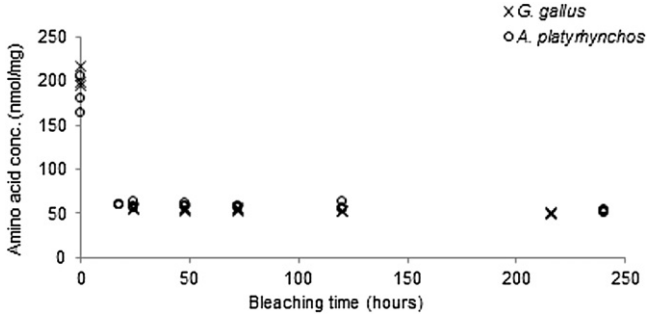

Fig. 3. Isolation of intra-crystalline proteins in two species by bleaching with NaOCl. The sharp drop in amino acid concentration upon exposure to bleach represents the oxidation of residues exposed to the external environment. The plateau in concentration shows that some of the amino acids are not exposed to the bleach; this suggests the presence of an intra-crystalline protein fraction (e.g. Penkman et al., 2008).

Ultraflex III MALDI-ToF (Matrix-Assisted Laser Desorption/Ionisation – Time of Flight) with the following parameter settings: ion source, 25 kV; ion source, 21.4 kV; lens voltage, 9 kV; laser intensity, 35–40%; and mass range, 800–4000 Da. Peptide masses below 650 Da were suppressed. Final mass spectra were externally calibrated against an adjacent spot containing 6 peptides (des-Arg^1-Bradykinin, $m/z = 904.681$; Angiotensin I, 1296.685; Glu^1-Fibrinopeptide B, 1750.677; ACTH (1–17 clip), 2093.086; ACTH (18–39 clip), 2465.198; ACTH (7–38 clip), 3657.929). FlexAnalysis software 3.3 (Bruker Daltonics) was used to baseline subtract, normalize spectra and determine peak m/z values and intensities in the mass range of 800–4000 m/z.

2.3. Taxonomic identification

Two separate approaches were developed for identification of eggshell fragments. The first is based on identification of potentially diagnostic peptide masses. For the vast majority of species no sequence data were available; potentially diagnostic peptide markers were identified by screening peptide masses found in each species against the entire reference collection ($S/N \geq 6$). This approach provided a reference list of peptide masses which were potentially useful as taxonomic indicators, although the level of resolution achieved varies between markers (Section 3.1, Table 1).

Rather than identifying specific markers, the second approach uses the whole list of peptide masses from reference spectra to identify the species represented in a sample (e.g. Henzel et al., 1993; Hollemeyer et al., 2007, 2008; James et al., 1993; Pappin et al., 1993). Previous ZooMS analysis of mammal, bird and fish bone has shown that a methodology based on MALDI-TOF mass spectrometry and PMF can taxonomically identify heavily fragmented archaeological biomaterial (Buckley et al., 2009, 2010; Richter et al., 2011).

For all archaeological samples, matching of mass spectra to species based on comparison with reference spectra was performed using an in-house Microsoft VB application (ChickenHawk) (R. Allen, University of York – see Supplementary information for a description of the software). This software searches a reference database constructed of known peptide masses and reports both the number of matches between observed peptide masses and data in the reference collection, and the percentage of peaks observed in each species which are observed in the sample (an example is provided in Supplementary information). It also screens the data for the presence or absence of potentially diagnostic peptide markers. The resolution can be limited by the extent of the reference collection, which varies by taxonomic group; ChickenHawk will identify which species in the reference collection is the closest match, but cannot always extrapolate this into definite species identification. Identification to family or order is more realistic in some cases. This situation, which is analogous to conducting zooarchaeological analysis with a limited reference collection, will improve as the range of bird species analysed increases. The major advantages of this approach are that it is applicable to all species, is very fast, and can be very accurate if the relevant sections of the reference collection have good coverage. In order to test the accuracy of the software, data representing all species in the database were screened; in all cases, ChickenHawk provided the correct match.

Table 1
Selection of peptide markers obtained by screening the reference database, highlighting the variability in the level of resolution achievable using this approach. These data represent an archaeological sample which contained chicken and goose eggshell. Convergent masses are often observed in diverse taxa, but these data can still constrain the possible range of species present. The entire list of markers ($n = 227$) is provided as Supplementary information.

Peptide m/z	Marker ID
1042.6	Galliformes/Corvidae
1247.6	Anseriformes
1265.6	Goose/swan
1309.7	Galliformes/Corvidae/Scolopacidae
1348.8	Chicken/duck
1372.6	Goose/Aythya/Accipitriformes
1392.8	Anseriformes
1528.7	Goose/Aythya
1688.7	*G. gallus*
1722.8	Goose
1734.9	*G. gallus*
1774.8	*G. gallus*
1808.9	Galliformes
2051.9	Anseriformes
2392.2	Anseriformes

3. Results

3.1. Taxonomically diagnostic peptides

Potential markers were isolated by screening the entire dataset. In some cases, peptide masses converge with those observed in other species. Therefore, these should not be considered taxonomically diagnostic, but can constrain the possible range of species observed. A sample list of potentially diagnostic markers is provided below (Table 1); the entire set is provided as Supplementary information.

3.2. Peptide mass fingerprints

Peptide mass fingerprints (PMF) were successfully obtained for all species analysed. These provide the main means of identifying eggshell fragments in most cases. All of the PMF data are included as Supplementary information. Examples of some of the spectra obtained are presented (Fig. 4). The level of resolution achievable using this technique varies between taxa. For example, although PMF allows distinction between ducks and geese, confident identification of different species or of domestic and wild types is not currently possible.

3.3. Hungate case study: introduction

Hungate is located near the centre of York, abutting the north bank of the River Foss (Fig. 5). The site is large, and the quality of preservation varies between different areas depending on soil type and chemistry, and on hydrological conditions. In much of the site organic preservation is restricted to deposits located below the mean water table. In addition to a huge volume of animal bone, frequent occurrences of preserved wood, leather, mollusc shell, eggshell, coprolites and other organic materials have been recovered. In most cases eggshell was recovered by 5 mm and 1 mm sieving, which YAT excavators performed routinely on most types of deposit. Most of the contexts described below are provisionally assigned to Viking Age (late 9th century–mid 11th century) activity (unless stated otherwise), but this information is incomplete at present (T. Kendall, pers. comm.).

3.4. Results of case study

All samples were identified using both of the systems described in section 2.3. Multiple eggshell fragments ($\leq$5 fragments) were routinely included in a single analysis in order to facilitate analysis of all recovered fragments; the identification techniques described above can distinguish different species in a single sample (Supplementary information). The results of the case study are

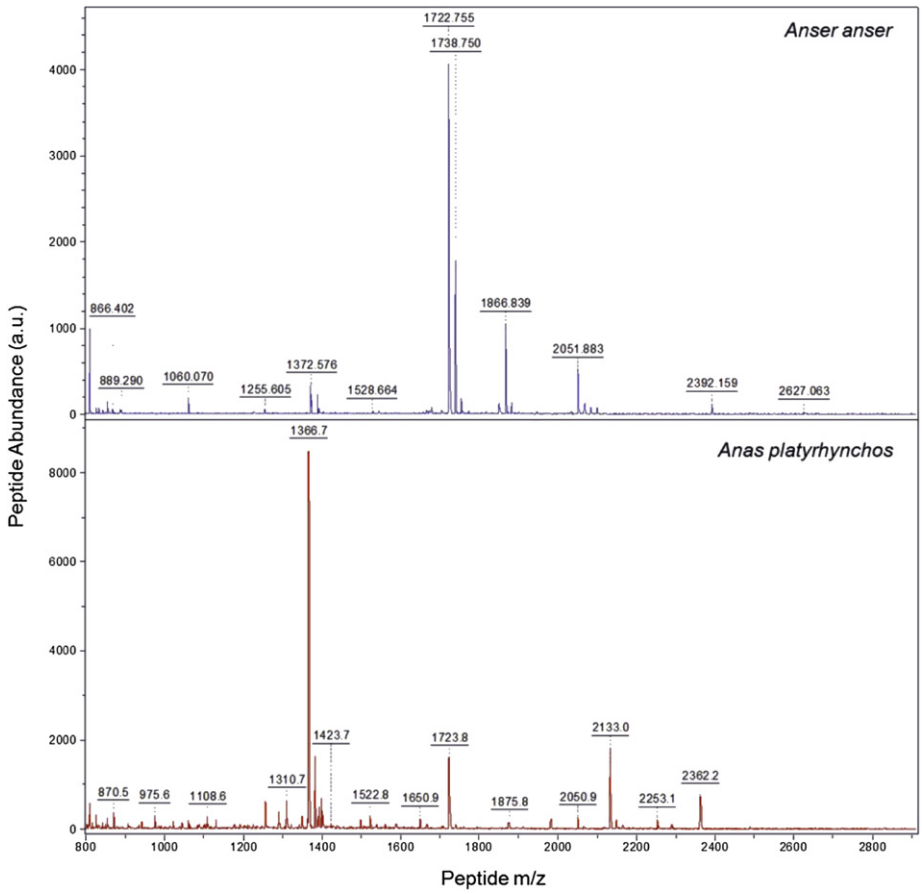

Fig. 4. Example selection of mass spectra obtained from the reference collection. The species represented are greylag/domestic goose (*Anser anser*) and mallard/domestic duck (*Anas platyrhynchos*). Data and mass spectra for all other species are included as Supplementary information.

presented below (Table 2). As a single sample may contain multiple eggshell fragments, this approach allows the *minimum* percentage representation of any given species to be calculated. Although this technique produces qualitative data, the method of quantification used in interpretation only requires that the presence of any given species is detected (see Section 4.2). All context information was provided by T. Kendall (Field Officer for Hungate). All contexts from which eggshell was recovered are described, even if attempts to identify the eggshell were unsuccessful. Identifications were achieved for 35 out of 39 contexts (a success rate of 89.7%). Some of the contexts from which no successful identification was made were relatively young (Table 2): this suggests that the failure to recover any identifiable peptides relates to something other than age, perhaps such as burning events. It is also possible that in some instances small pieces of other materials (e.g. small pieces of plaster, mollusc shell) can be mistakenly identified as avian eggshell (T. Kendall, pers. comm.). Unsuccessful identification was due to poor quality spectra, rather than peptide mass fingerprints which were not represented in the reference collection.

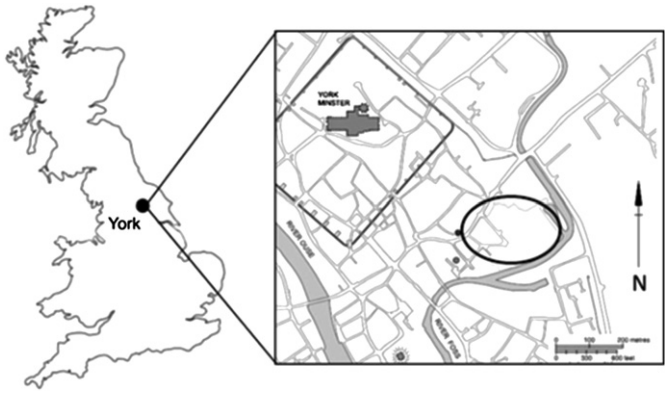

Fig. 5. Location of Hungate site. Reproduced courtesy of York Archaeological Trust; based on the Ordnance Survey mapping © Crown copyright.

4. Discussion

4.1. ZooMS: the technique

The preceding sections have described the development of a rapid and reliable technique for analysis of archaeological eggshell assemblages. The major current limitation of this technique is the paucity of readily available reference material: some species are represented in the reference collection by a single eggshell fragment. The low number of specimens representing some species requires justification. While far from ideal, this is a problem which is not immediately surmountable: British law prohibits collection and ownership of wild bird eggshell, and museum stores are usually the only source of reference material. In well-represented domestic species, there is very limited variability in the range of peptides recovered (Table 3), although there can be considerable variability in the actual concentrations of these peptides.

As there is no reason to believe that protein concentration is homogenous in any part of the eggshell, the variability in concentration is unsurprising. The lack of variability in the range of

Table 2
Results of Hungate case study. All fragments recovered were analysed. X indicates the presence of a species in the deposit.

Context	Description	Date	Fragments	Chicken	Goose	Proportion
48310	Pit backfill (lower level)	14th C.	2	X		
48314	Pit backfill	14th C.	12	X		
48709	Cess-pit backfill	Unknown	5	X		
48716	Cess-pit backfill	Unknown	2	X		
48780	Pit backfill	11th–12th C.	7	N/A	N/A	
49087	Wattle pit lining	Unknown	1	X		
49223	Cess-pit backfill (upper)	Viking Age	2	X		
49478	Fill of stake hole	Unknown	60–70	X		
49480	Rubbish pit backfill	Unknown	2	X		
49487	Cess/refuse pit backfill	Unknown	40–50		X	
49494	Pit backfill	Unknown	4	X	X	75% goose
49509	Cess-pit backfill (upper)	10th C.	2	X		
49599	Levelling deposit of occupation layer	Unknown	>1000	X		
49645	Cess-pit backfill (also domestic waste)	10th C.	3	X		
49646	Pit backfill (cess & domestic waste)	Late 10th C.	50–60	X		
49671	Cess-pit backfill	Pre-late 10th C.	150	X		
49720	Construction/foundation backfill	Unknown	4	X		
49731	Construction backfill	Unknown	12	X		
49810	Infill/packing material between steps	Unknown	6	X		
49817	Use backfill of cess-pit/latrine	17th C.	1	X		
49827	Backfill of sunken-feature building	Unknown	2		X	
49854	Rubbish pit backfill	Unknown	3	X		
50551	Dump deposit	Mid/late 10th C.	10		X	
50834	Construction backfill	13th C.	6	N/A	N/A	
50839	Pit backfill	Viking Age	2	X		
51266	Cess-pit use deposit	10th C. or older	80	X		
51435	Pit backfill/use deposit (lower)	10th C.	1	X		
52192	Road preparation level	Unknown	1	N/A	N/A	
52300	Pit backfill	Unknown	13	N/A	N/A	
52438	Banded deposit overlying cess-pits	10th C.	60	X		
52444	Cess-pit/domestic waste backfill	10th C.	60	X		
52852	Demolition backfill	10th C.	70	X		
52960	Refuse pit backfill	Unknown	50	X		
83328	Floor surface	13th–14th C.	66	X		
83350	Floor surface	13th–14th C.	6	X		
83460	Floor surface	13th–14th C.	66	X		
83461	Floor surface	13th–14th C.	108	X		
83471	Floor surface	13th–14th C.	26	X		
83548	Floor surface	13th–14th C.	16	X		

peptides recovered provides some confidence that small sample sizes do not lead to unrepresentative reference spectra. It also highlights that this technique is qualitative rather than quantitative. Quantifying the number of shell fragments in a deposit which represent any particular egg is usually all but impossible; this technique deals in the presence or absence of particular peptides (and thus taxa), rather than direct quantification.

We have not analysed any eggshell older than Viking-age York (<1200 yrs) but the high concentration of some peptides recovered from this material suggests that the ZooMS method might be applied to considerably older material (Fig. 6).

4.2. Discussion of Hungate results

Bird bones account for between 1.9% and 7.5% of the animal bones recovered from Anglo-Scandinavian sites in York (O'Connor, 1989, 2000). The extent to which this reflects taphonomic differences in preservation and recovery of bird bone, which is generally smaller and lighter than mammal bone, is unknown. However, it is clear that birds contributed significantly to the diets of people in York during this period, and it is probably safe to assume that these numbers underestimate their actual contribution. The majority of the recovered bird bones represent domestic chicken, while goose and duck bones are also reasonably abundant: it has been cautiously proposed that these largely represent domestic birds (O'Connor, 2000).

Quantification of the eggshell data presents a challenge, as there is no way of knowing how many eggs are represented in any given eggshell assemblage. In this context, the proportion of fragments representing any given species in an assemblage is meaningless. A more appropriate means of quantification is to calculate the proportion of contexts in which each species occurs. The current eggshell evidence suggests that domestic chicken (present in 89.7% of contexts analysed) provided the majority of eggs used at the site during this period (Table 2). There is also a small contribution of goose eggshell (present in 10.2% of contexts analysed) (Table 2). Although this technique cannot distinguish domestic and wild types, these results are consistent with domestic species providing the vast majority of the eggs consumed in the city, with at most a minimal contribution from wild species.

Non-domestic species are also represented among the bones (O'Connor, 1989, 2000). As with the biomolecular technique described here, it can often be hard to distinguish between the bones of different anseriform species using commonly available morphological techniques (O'Connor, 2000). Non-domestic species

Table 3
Variability in *m*/*z* values of selected peptides observed in modern samples of known species.

Peptide (*m*/*z*)	Species	Standard deviation	*n* (replicates)
1042.561	*G. gallus*	0.0432	1036
1150.608	*G. gallus*	0.0413	948
1293.671	*G. gallus*	0.0379	861
1366.618	*G. gallus*	0.0183	151
1418.768	*M. gallopavo*	0.0312	42
1615.816	*S. camelus*	0.0259	15
1800.889	*U. aalge*	0.0350	11
1872.875	*G. gallus*	0.0724	618

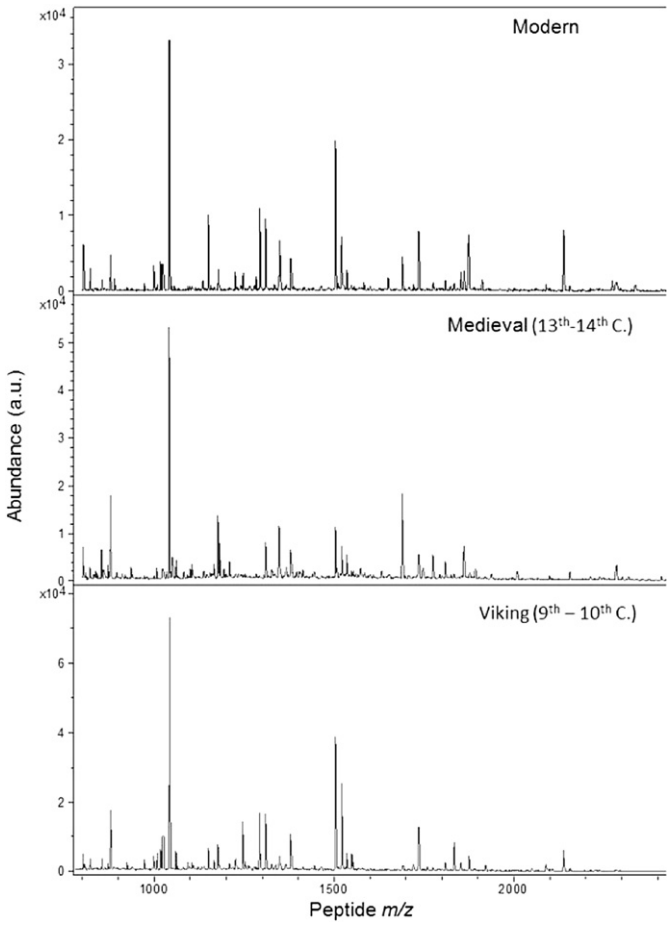

Fig. 6. Peptide concentrations observed in single samples of modern, medieval and Viking Age chicken eggshell. The range of peptide abundances recovered from all three ages varies considerably: the Viking Age material varies by orders of magnitude (Supplementary information). For the Viking Age material, the highest concentrations recovered are presented here. The other spectra were selected randomly from the data.

recovered from Anglo-Scandinavian sites in York include a range of wetland birds such as wild ducks and geese, cranes, and a range of waders: these are commonly winter-flocking species, and were most likely exploited during winter when farming activities placed less demands on time (O'Connor, 2000). Other species represented in the bone assemblages from Anglo-Scandinavian York include cliff-nesting seabirds (razorbill, guillemot), woodland species (woodcock, wood pigeon), and moorland species (golden plover, black grouse) (O'Connor, 1989).

There was not a single instance of eggshell recovered from Hungate which was shown to represent any species which is not known to have been kept domestically in the city (although this cannot be conclusively stated for wild geese – see above) (Table 2). In his analysis of the animal bone recovered from Anglo-Scandinavian deposits in York, O'Connor suggests that egg production was probably the main focus of chicken farming during this period (O'Connor, 2000). The lack of demonstrably wild species in the eggshell assemblage supports the idea that wild-fowl may have been a focus of exploitation during winter (i.e. outside of their breeding season); during the period when eggs were available, people would be occupied with farming activities (O'Connor, 2000). The eggshell results from Hungate begin to suggest that domestic species were the only source of eggs exploited in the city during this period. This is a preliminary interpretation, based upon analysis of a single site. Future research will aim to further develop understanding of the use of bird eggs in Anglo-Scandinavian York, and beyond. Current research is also analysing eggshell assemblages from contemporaneous coastal sites, which are likely to contain a higher proportion of wild bird eggshell.

4.3. Value of museum collections

There have been sustained curatorial efforts to establish the scientific research value of museum egg collections in recent years (e.g. Russell et al., 2010). One recent study urged caution when considering the utility of museum eggshell for proteomic research, as some proteins found in modern shell were not recovered from museum material (Portugal et al., 2010). However, this study did not account for the presence of distinct inter- and intra-crystalline protein pools: it is probable that the proteins which were missing are not commonly located in the intra-crystalline fraction, and/or are particularly susceptible to rapid diagenesis. None of the missing proteins belonged to the C-type lectin family, which has been observed in all species in this analysis for which proteomic data are available, and which play major roles in deposition and regulation of the eggshell (Gautron et al., 1997, 2001; Keung and Azari, 1982; Portugal et al., 2010). For the purposes of analyses such as this one, in which recovery of the full suite of proteins found in modern eggshell is not essential, museum stores represent a unique research resource.

5. Conclusions

Due to the difficulty of assigning taxonomic status to preserved eggshell fragments, there is a major disparity between the prevalence of eggshell in the archaeological record and the frequency and depth of discussion of it. A rapid, reliable and robust system for taxonomic identification of preserved eggshell fragments using protein mass spectrometry is designed to be able to cope with large volumes of material, and its resolution will increase as the reference collection increases in depth. The Hungate case study demonstrates that this new technique is viable for use on archaeological material: the development of this technique opens up archaeological eggshell to new interpretation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jas.2012.11.007.

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