

RESEARCH ARTICLE



Species identification using ZooMS, with reference to the exploitation of animal resources in the medieval town of Odense

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ABSTRACT

ZooMS (Zooarchaeology by Mass Spectrometry) is increasingly being used as a method for species identification of archaeological and historical remains. The method identifies species from the peptide mass fingerprint of extracted collagen – the principal protein of bone, ivory, dentine, leather, and parchment. ZooMS has the advantages that it is a fast and simple method, that requires only small sample sizes or even non-destructive sampling. The taxonomic resolution of the method varies, but ZooMS is diagnostic for most domesticated animals and for the relatively depauperate Scandinavian fauna, although some groups (seals, martens) cannot be resolved, and it cannot discriminate some domesticates (dog, cattle) from their wild counterparts. In this article, we overview the method and demonstrate the value of ZooMS and illustrate our points via a case study of 20 samples from 12th to 14th century layers in the Danish medieval town of Odense. Four artefacts were tested by a non-destructive eraser technique because of their uniqueness, but only one could be identified. The remaining 16 were identified following destructive analysis of the sample, one sample could not be identified.

Through the identification of a gaming piece as walrus tusk the analysis demonstrated the long distance trade networks of Odense and the pursuit of some inhabitants for luxury products and high living standards. Conversely, the species identification of combs showed that the medieval comb maker would use the resources immediately available to him to create an affordable everyday object rather than rely on imported antler.

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Introduction

The study of animal remains such as bones, skin, and fur in an archaeological context provides insights into past relationships between animals, people, and the environment. Because of the mutual nature of these relationships, animal remains in context have been used to address a wide range of aspects of the human past as amongst many others diet, resource exploitation, animal domestication, economy, environment, trade networks, and cultural identity, and the study is relevant across prehistoric and historic periods, settlement types, and geographical regions (Steele 2015). Identifying the species of animal remains is one of the key prerequisites for discussing such aspects of human culture.

This article explores the protein fingerprinting methodology of ZooMS (Zooarchaeology by Mass Spectrometry, Buckley *et al.* 2009) which uses

amino acid sequence variation in the dominant structural protein, type I collagen, which is abundant in bone, skin, and tissue, for species identification in Scandinavian archaeology. Since its introduction, the method has reached maturity and is becoming increasingly popular within archaeology as it is a cheap, easily applicable, and minimally or even non-destructive method for species identification (Fiddymment *et al.* 2015, Coutu *et al.* 2016). Moreover it has been demonstrated to be an excellent method for screening large bone assemblages for specific species (Welker *et al.* 2016).

In this article, we will introduce ZooMS and then present a case study in which the method has been used as part of an analysis of the 12th to 14th century animal resources from the Danish town of Odense.

The Scandinavian record

In many sites in Scandinavia, animal bones and skin (rich in collagen) are common finds whereas fur and woollen textiles (rich in keratin) are rare. In fact, animal bones (with waterlogged wood) are the most abundant organic materials that we come across in urban excavations. They are often present in more or less every cultural layer and deposited in every conceivable way; from intact buried animals to fragments in the form of dietary remains, waste from butchering, worked bone or antler, semi-manufactured artefacts and debris from production, finished, discarded or lost artefacts, or reused animal bones in structures as fills.

Because of its abundance and witness of multiple processes, bone holds great interpretive potential, but it is also one of the most challenging to handle. This is not only due to the complicated processes related to its use and deposition but also because of the circumstances that applies to most excavations in Denmark. The majority of the archaeological excavations in Denmark are rescue excavations that are conducted within the framework of the Danish Museum Act part 8.¹ This entails economic restrictions and specific guidelines with regards to the analysis of zooarchaeological material.² In practice this implies that it is impossible to collect the preserved bone material in its entirety. The analyses that are carried out are mainly quantitative in nature, where the number of bones, species and sex identification, and their distribution over time are accounted for.

One could argue that collecting all animal bones from a site would be a senseless endeavour, since the excavated material already only represents a part of the original bone assemblage due to selection processes in the past and the following taphonomic processes (Orton 2012). An analysis of the zooarchaeological material from any archaeological site will always have to consider sample bias and fragmented material. This makes it even more important to consider all available information from the archaeological record. Only then it is possible to answer questions such as: What animals provided the raw materials for both food and manufacture of artefacts? Were they local or not? Were some animals preferred over others? And for what purpose? These questions are especially pertinent to the medieval town, since the use of animal resources are part of the economy that sustained life in the town and

therefore part of what characterises life in town. Describing these practices on best possible ground will enable us to understand the dynamics that constituted life in the town and ultimately what makes town life different from other forms of existence (Christophersen 2015). In order to answer any of these questions, it is necessary to identify the species of the zooarchaeological material *including* the material that is not identifiable through morphological characteristics. ZooMS offers to do so in a way that is affordable, reliable, and within the scope of the Danish Museum Act.

Species identification of animal remains

Species identification of animal bones has traditionally been performed by osteological examinations of the size, morphological characteristics, and surface features of bones that vary between species (O'Connor 2000). This can lead to the determination of the species of its origin by comparing the observed characteristics with characteristics on bones of known species origin from reference collections or animal bone atlases. These morphological species identification methods are especially valuable in that they often provide additional information to the species, such as the bone element, sex and age of the animal, pathology, traces of wear, and the preservation state of the object (Steele 2015). However, the success of osteological species identification depends on the preservation of the diagnostic characteristics of bones and the opportunity to identify them. Diagnostic characteristics may be lost due to, for instance, processing bones for consumption, working bone into artefacts, taphonomic processes such as weathering and gnawing or diagenetic processes following burial (Lee Lyman 1994). If some animal bones are more heavily processed than others, their importance may be overlooked as their fragments may be less recognisable. Even for well-preserved bones, both wild and domesticated species may display significant variations of bone elements within a species for instance between males and females and between different populations (Hillson 1992). This means that considerable expertise is required for reliable identifications, but also access to reference collections encompassing all such interspecies

variations. The last is further a challenge as some species are known to have changed morphologically over the past millennia and during domestication. Examples of this is a great diversity of horns in cattle, sheep, and goat after domestication and a shortening of the face region and jaws seen in for instance domesticated dogs and pigs (Clutton-Brock 1999).

When diagnostic features are not preserved, archaeologists are left with no species identification or identification to a higher taxonomic level, which can lead to large percentages of unidentified bones and bias the interpretations of a bone assemblage (Badenhorst and Plug 2011); indeed archaeologists include the term oviscaprid due to the difficulty of discriminating sheep from goat.

Over the past two decades, developments within biomolecular archaeology have resulted in a range of technologies which are applicable for species identification of archaeological material. Of these, the analysis of ancient DNA is probably the most well-known. DNA from a range of archaeological materials has been successfully extracted and sequenced both targeting short fragments of mitochondrial DNA using PCR (Polymerase Chain Reaction) and larger parts of the genome using Next Generation Sequencing (NGS) technologies. Such studies have provided species identifications of animal bone, skin, and hair (Fiddyment *et al.* 2015, Brown *et al.* 2016, O'Sullivan *et al.* 2016, Welker 2017). The great advantage of DNA studies is the high resolution which allows the distinction between even closely related animal species, and male from female, but also it can discriminate populations, and identify migrations (Cassidy *et al.* 2017, Librado *et al.* 2017). NGS analyses are still relatively expensive and time consuming and the success of aDNA analysis largely depends upon the preservation of DNA, a process which is still poorly understood (Smith *et al.* 2003, Kistler *et al.* 2017). However, materials with no preserved DNA may not be out of reach for species identification.

Over the past years, proteins have been demonstrated to persist for longer than DNA and can be recovered in environments from which DNA cannot be amplified (e.g. eggshell, Demarchi *et al.* 2016, and bone, Welker *et al.* 2015a, Westbury *et al.* 2017). In the case of skin capes from Danish bogs dating to the Iron Age (Schmidt *et al.* 2013), DNA identification failed, whereas a proteomic approach was able to provide species identifications of the skins. More

than this, proteins can be tissue and developmentally specific, and the study above provided additional information on the use of young (calf) skin for one of the capes (Brandt *et al.* 2014). Both NGS-based DNA approaches and proteomics methods are however relatively expensive and time consuming. In many cases, merely species identification can be the most relevant information for answering an archaeological question together with traditional zooarchaeological analysis such as quantification, animal size, element processing, etc.

ZooMS – Zooarchaeology by Mass Spectrometry

Whereas shotgun proteomics, as the name suggests, targets all proteins preserved in a sample, ZooMS usually targets the protein collagen, the most abundant protein in animals where it is found in connective tissues, bone, antler, teeth, and skin (Shoulders and Raines 2009). So far, 28 collagen types are described. Of these, type I collagen is the most abundant and found in various tissues including bone, skin, ligament, and tendon. It is a tough, insoluble protein, which is difficult to biodegrade if protected by mineral (bone, dentine, antler) or tanned (leather). Consequently it is the most common protein recovered from archaeological environments. In higher vertebrates, type I collagen consists of three polypeptide chains: two collagen $\alpha 1$ chains and one collagen $\alpha 2$ chain, which are coiled into an extended proline triple helix. Collagen forms a nanorope; the triple helices self assemble into larger collagen microfibrils, which aggregate to form collagen fibres (Shoulders and Raines 2009).

After collagen, the second most widely studied protein from archaeological remains is keratin, found in instance wool, hair, nail, hoof, beak, and feathers (Hollemeier *et al.* 2002, 2007, 2008, 2012, Solazzo *et al.* 2013, 2014, Solazzo 2017).

Sampling

When analysing archaeological material only small samples are needed. For example, 'empty' tubes used to process collagen for radiocarbon dating (Charlton *et al.* 2016) and eraser rubbings used to clean parchment (Fiddyment *et al.* 2015), have both been used. In the case of archaeological samples, 10-30 mg of

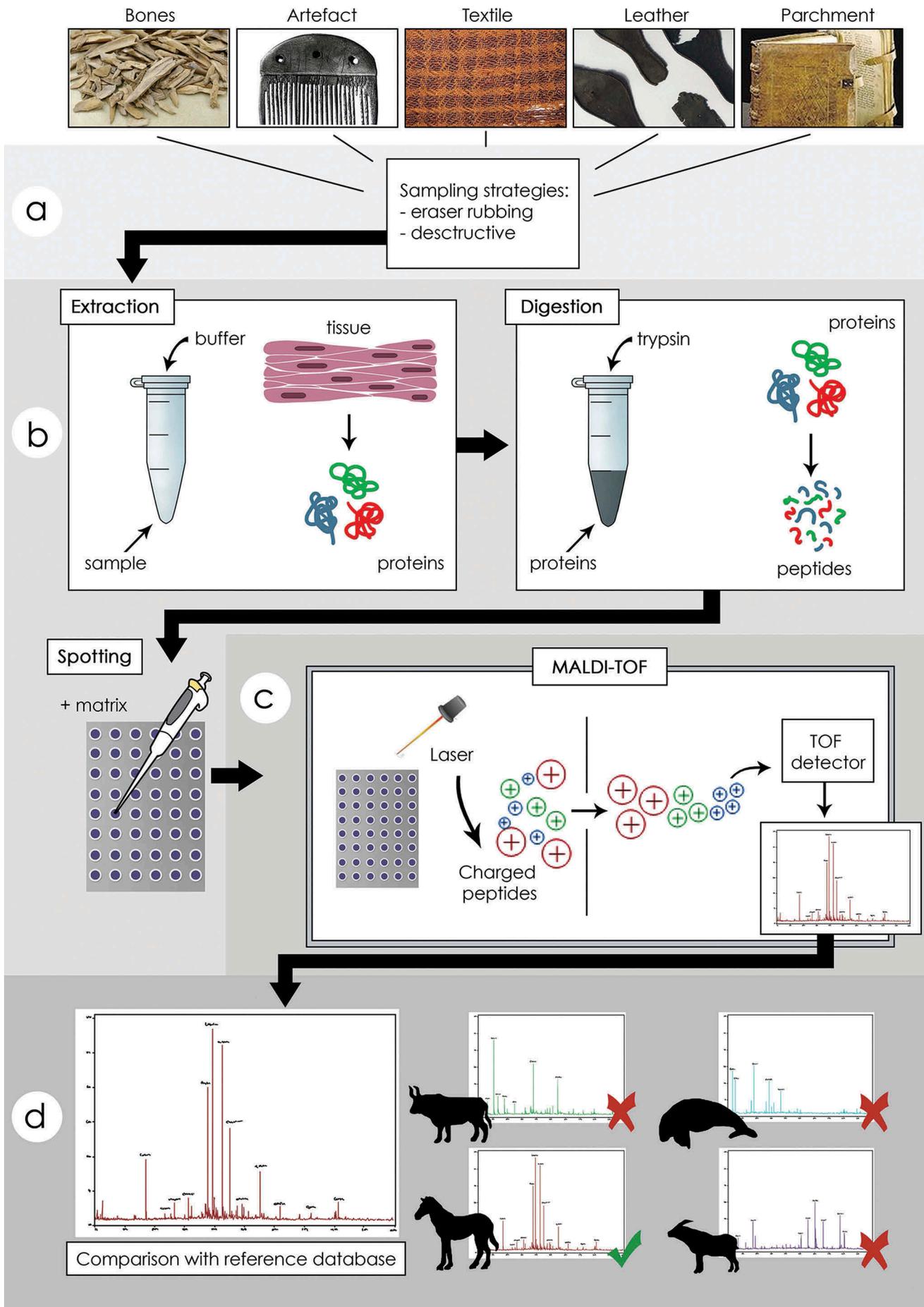


Figure 1. Flow chart of the ZooMS process. Graphics: Sidsel Frisch.

bone (depending on whether bone chips or bone powder is used) or pinhead of skin is sufficient (Figure 1(a)). Being able to sample non-destructively is crucial for getting access to materials from which a sample cannot be spared.

Extraction and digestion of collagen

The extraction protocol varies according to material and strategy (Figure 1(b)). For bone samples, a destructive demineralisation can be applied to dissolve the bones mineral component using hydrochloric acid, which leaves a collagen pellet that can be gelatinised using ammonium bicarbonate (Buckley *et al.* 2009). An alternative and non-destructive approach has been developed (Van Doorn *et al.* 2011), which avoids the demineralisation step, extracts sufficient collagen for ZooMS, and leaves the sample undamaged.

Regardless of the extraction method, the subsequent digestion is usually the same. The solubilised collagen (gelatin) is cleaved using the enzyme trypsin into shorter chains of amino acids (peptides) at lysine and arginine residues. Together lysine and arginine represent about 10% of all the residues in collagen, but they are not evenly distributed over collagen, thus although the average length of a chain will be about 10 residues, resulting peptides will have a varying length, composition, and mass. It is these masses, when known to vary between animal species, due to differences in the amino acid sequence, which enable species identification. However, the low degree of sequence variation in collagen, and the degree of structural constraint means that different peptides can share the same mass. The peptide digests are desalted, typically using C18 columns (ZipTips) and eluted in (TFA) acidified acetonitrile. It is possible to fractionate the eluent from the ZipTip by using different concentrations of solvent. 1 µl of each sample is spotted in triplicate onto a plate (both disposable plastic and reusable stainless steel can be used) to which 1 µl of matrix is added, and the two co-crystallise.

Analysis

The mass of each peptide is measured using Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF) (Figure 1(c)). A

laser is targeted at matrix crystals formed as the sample dries on the plate. Using a compatible wavelength to the matrix, the absorbed energy causes the (acidic) crystals to volatilise transferring charge (a single proton) to some of the co-crystallised peptides. An electric field accelerates the volatile charged peptides down a vacuum tube, towards an ion mirror which doubles the length of the flight path and helps focus the ions towards the detector. As all peptides carry a single charge, the smaller and lighter peptides travel faster down the tube than the larger and heavier ones. The time of flight to reach the detector can be used to estimate the mass of the peptide and the resulting peaks generated on a spectrum reflect the mass and the intensity of the detected collagen peptides. Each sample therefore gives a fingerprint of masses of the constituent collagen peptides.

Identification

It is typical to run triplicate spectra, as differences in co-crystallisation impact upon system performance. The three spectra resulting from the triplicate spots of each sample can be averaged and analysed using software tools such as mMass (Strohalm *et al.* 2008).

Masses which represent collagen peptides of different masses are recognised. The masses are then compared to a list of collagen peptide masses from the species that have been analysed, or predicted from sequences. By comparing the masses, it will be possible to rule out species (Figure 1(d)). The more peaks recognised on the spectra, the better the chance for a specific identification. In the ideal situation, the peaks will represent masses that are diagnostic for only one species. Some species are however so closely related that it will not be possible to distinguish them (Buckley *et al.* 2011, Coutu *et al.* 2016). Mammals have been the primary focus for ZooMS identifications, but the reference database also includes markers for fish (Richter *et al.* 2011) and markers for eggshell from birds (Stewart *et al.* 2013, Presslee *et al.* 2018) and is constantly expanding.

Depending on the extraction method, the entire analysis from sampling to analysis can be carried out in few days. Altogether ZooMS therefore represents a minimally or even non-destructive, cheap, easily applicable, and fast method for species

identification of archaeological remains rich on collagen or keratin.

Case study: animal remains from medieval Odense

The site

From May 2013 to September 2014 a large rescue excavation prior to construction work took place in Odense; the third largest town in present day Denmark.

Odense is first mentioned in a letter in 988.³ Also, in the 10th century, a Viking ring fortress was built in Odense, in the 1040s it is possible that minting took place, and in 1086 King Canute (the Holy) was killed before the altar in St. Albany Church and later canonised (Christensen 1988). These events all suggest that Odense was a significant settlement already in the 11th century although it has been difficult to characterise the settlement further through the archaeological record.⁴ By the 13th century, Odense acquired market rights and the archaeological and written records testifies to a vibrant and growing town that maintains its position as one of the most important towns in Denmark throughout the Middle Ages.

An area of 2500 m² with approximately 4300 m³ of cultural layers in the central part of the medieval town

Table 1. Number of animal bone fragments from the 2013–2014 excavation in Odense (OBM9776) and their distribution through time.

Period	No. of fragments
–1100	848
12th Century	12.031
13th Century	30.404
14th Century	31.956
15th Century	8.843
After 1500	100
In total	84.182

was excavated. A coherent area of that size and location had prior to this never been excavated in Odense. All though the area had been heavily truncated by modern construction activity the preservation conditions for wood, bone, and other organic material were surprisingly excellent. Both an extensive finds assemblage and well-preserved structures such as stalls, houses, stables, latrines, paths, roads, fences, manure heaps, and much more was brought to light. This presented a unique opportunity to study the period from the 11th to 16th century CE in Odense in detail (Figure 2).

Zooarchaeological results

A total of 84,182 fragments of animal bones were recovered from the cultural layers – all as the result of an extensive sieving procedure (Table 1). Of these it was possible to identify the species or family level of 40,913 fragments which illustrates the challenges in

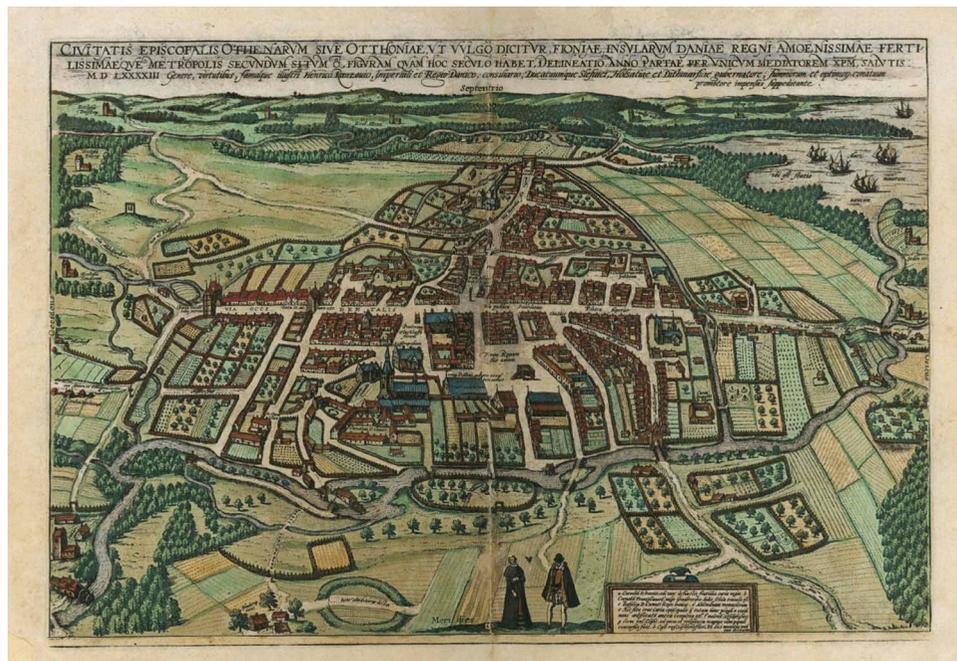


Figure 2. Odense around 1593 AD – Braun & Hogenberg *Civitates orbis terrarum* Vol. V.

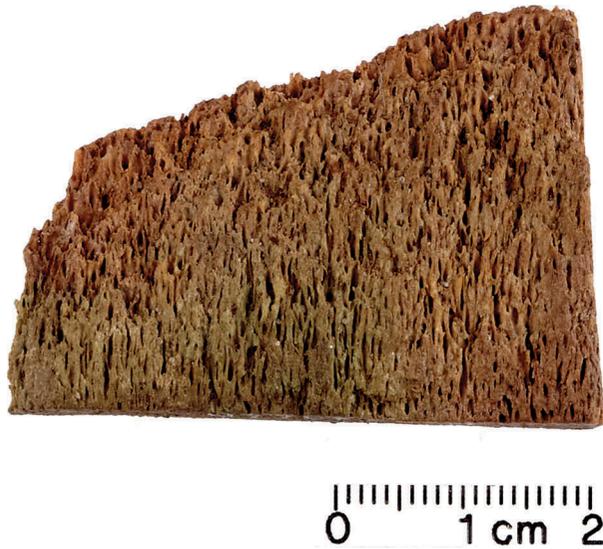


Figure 3. Photo of a piece of worked right whale bone (x5850). Photo: Nermin Hasic, Odense City Museums.

working with this often fragmented material. In spite of only half of the bones being identified, the results of the excavations and the zooarchaeological analysis demonstrate that a wide range of domestic animals such as horse, pig, cattle, sheep, and goat have been an important contribution to life in town from the 11th to 16th centuries (Østergaard 2016). On each stage of their life-history, these animals would be an almost indispensable resource acting as draught animals, providing milk, meat, and raw materials such as leather and bone for manufacturing various objects.

A total of 208 artefacts were produced of either antler or animal bone. Amongst these were semi-manufacture, production waste, needles, dice, combs, mounts, gaming pieces, handles, and a number of artefacts that could not be identified apart from the material; bone or antler. Forty of the 208 artefacts were identified to species level through morphological traits – most of these to red deer antler. 26 artefacts were identified as either a large or a small mammal. Amongst the artefacts for which the raw material could not be identified, 20 were selected for further analysis. These artefacts were chosen because they had a solid relation to an archaeological context and because this context was well-defined both stratigraphically and with regards to their interpretation. In addition, some were selected because of their unusual appearance (e.g. Figure 3). Finally, 13 samples were included because they were interpreted as being either combs, parts of combs, semi-manufacture, or production waste from comb-production. The artefacts were

moreover chosen so they spread over the 12th, 13th, and 14th century as one of the research questions was whether the selection of raw material for combs had changed over time. Another question was whether antler used for comb production derived from local animals from medieval Denmark, or from imported raw materials (Roesdahl 1999, Linaa 2015). A recent study of comb making in Viking Age Ribe, Århus and Aggersborg (Denmark) successfully used ZooMS to identify the raw material used as mainly cervid (red deer, roe deer, and reindeer, Ashby *et al.* 2015, 679–704). Also, Von Holstein *et al.* (2014) used ZooMS and DNA to explore evidence of trade with Scandinavia in pre-Viking Scottish combs. The number of samples from Odense was too small to give a representative picture of the development and the characteristics of use of animal resources and the analysis served therefore as a pilot project.

ZooMS analysis

For 16 of 20 bone samples, ZooMS was carried out using demineralisation according to Buckley *et al.* (2009) and for four samples using the non-destructive sampling with eraser (Fiddymont *et al.* 2015). Non-invasive sampling removes collagen using the triboelectric effect and was chosen, because it avoids the need to take a physical sample, when sampling complete or unique artefacts.

Table 2. Results of ZooMS identifications on 20 samples from the 2013–2014 excavation in Odense (OBM9776).

Specimen no.	Sampling technique	
	Destructive (Buckley <i>et al.</i> 2009), Non-destructive (Fiddymont <i>et al.</i> 2015)	Species ID
OBM9776		
x1276	Destructive	Cattle
x3025	Destructive	Bovid/cervid
x3116	Non-destructive	No ID
x3595	Non-destructive	No ID
x4685	Destructive	Bovid/cervid
x4715	Destructive	Bovid/cervid
x4974	Non-destructive	Bovid/cervid
x5559	Destructive	Cattle
x5651	Destructive	No ID
x5794	Non-destructive	No ID
x5850	Destructive	Right whale
x5864	Destructive	Cattle
x5875	Destructive	Red deer/fallow deer
x5953	Destructive	No ID
x6732	Destructive	Sperm whale
x7341	Destructive	Atlantic walrus
x7378	Destructive	Pig
x7564	Destructive	Horse
x7576	Destructive	Horse
x852	Destructive	Bovid/cervid

Results

Identification to species was possible for nine of 20 samples. Five samples failed to yield any identifications, while five samples were identified to the level of bovid⁵ or cervid⁶ and one to red deer or fallow deer (Table 2).

The possibility of identifying a sample depends first of all on the presence of collagen in the sample. It moreover requires that there are known differences between species and that peptides with these diagnostic masses are preserved. Not all the diagnostic peptides are identified in every sample and therefore identification will often be limited to a higher taxonomic level. To illustrate this, we have chosen the example of the whale, for which the 1682 peak is not shared with any other species. This provides a unique identification of the artefact to right whale (Buckley *et al.* 2014) (Figure 3).

Another example is the identification to a larger group of bovids and cervids present in the database. For OBM9776 x852, the presence of the peak 1427 m/z is shared between Bovidae such as sheep, goat, cattle, and gazelle and Cervidae such as reindeer, roe deer, fallow deer, red deer.⁷ Therefore, if there are no other peaks present on the spectra that differ between these species, the identification will be bovid/cervid. However, the peptide with the weight of 1427 differs from the peptide found in for instance pig, dog, and marine mammals, which after all excludes a range of species. If no diagnostic peaks are present, the result will be No ID.

The combs

The results of the pilot ZoomS analysis were somewhat surprising. As expected the medieval combs were primarily from animal bone. Generally it is widely accepted that medieval combs or combs manufactured in the 13th century and later are made from bone rather than antler

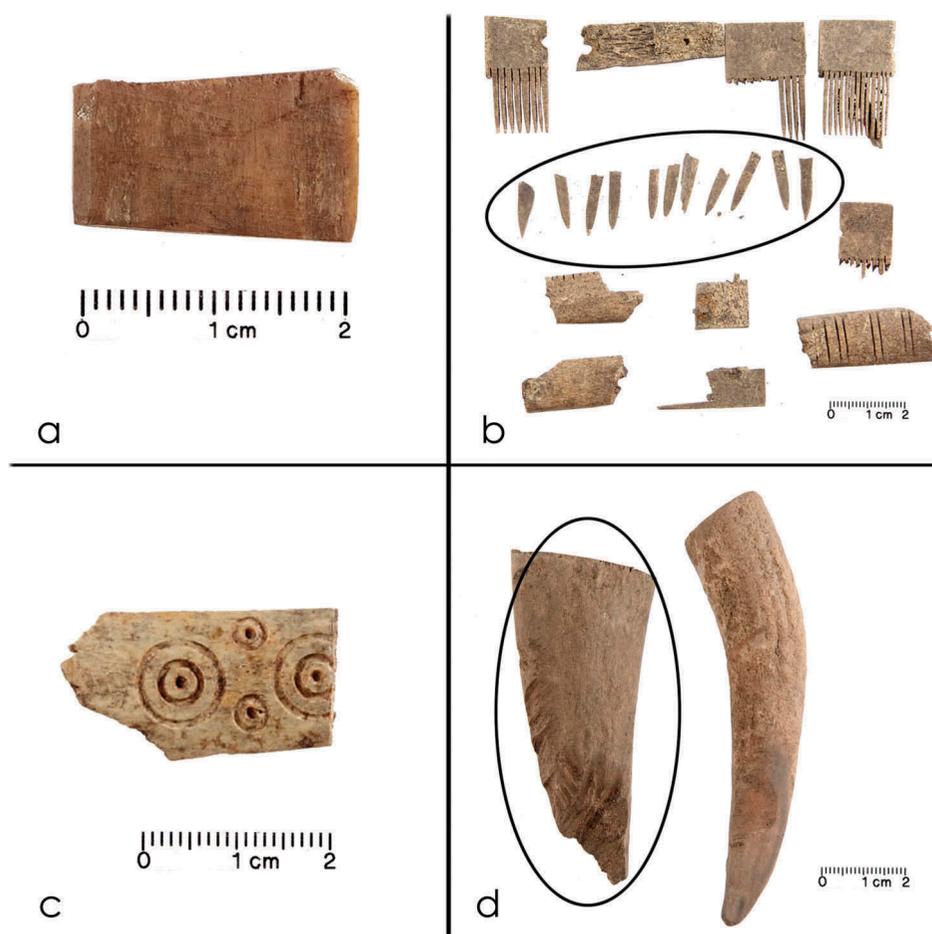


Figure 4. A selection of the analysed items. A: Horse (x7564), B: Pig (x7378), C: Bovid/Cervid (x4715), and D: Red deer/Fallow deer (x5875). Photo: Nermin Hasic, Odense City Museums.

(Øye 2005, Ashby 2009). The surprise was rather the *diversity* in the choice of species used for comb manufacture (Figure 4). For those parts with a positive identification, beyond bovid/cervid, the ZooMS analyses showed that cattle was the primary source for connecting plates (x5559, x1276) whereas horse, pig, cattle, and even bone from sperm whale have been used for making tooth plates (x7378, x7564, x5864, x6732). There was no positive identification for reindeer, roe deer, fallow deer, or red deer in any of the artefacts that with certainty could be related to comb production.

The presence of sperm whale (identified by key marker 2133 (Buckley *et al.* 2014)) was unexpected. Sperm whales are rare guests in the waters around Funen, but in the present day there are regular beachings of sperm whales on the west coast of Jutland, which might also have been the case in the Middle Ages. The beached whale belonged to the king, but locals were allowed some parts of it (Hybel and Poulsen 2007, 55). Maybe this was how the sperm whale bone ended up in Odense, brought there by a visitor or a merchant from Jutland. It does not seem that the comb maker distinguished between raw material from either small (pig) or large (horse, whale) animals. Horse bone was a very rare find amongst the animal bones (0,1%) in Odense and the main part of that material was represented by a complete horse that had been buried in a landfill area sometime during the 14th century (Østergaard 2016). The bones of this horse had cut marks which suggest that it had been skinned. Horses were mainly used for riding or as draught animals in the medieval period, but their skin would be used for leather. Finds of production waste from horse bone in Århus and Ribe suggest that it was not unusual to use horse bone in comb making in the Viking and medieval period (Møhl 1971, Enghoff 2006).⁸ In the aforementioned study from 2015, horse was found in a finished comb from Århus (Ashby *et al.* 2015, 690). In spite of the complicated procedure with removal of the flesh, etc. the examples show that horse bone may have played a larger role as raw material than the bone assemblage from Odense alone suggested.

With some precaution in regards to the small number of samples we may conclude that the medieval combs are a product of the town to a much larger degree than the Viking Age comb (Larsen 2005, Frandsen 2006, Ashby *et al.* 2015). It was possible to procure the raw material within the town limits including animals that

would probably not be used for dietary purposes – the horse. Antler was still used in comb production, as contemporary antler production waste from the area indicate, but it is most likely only from local red or fallow deer. The analysis suggests that at least half of the objects with relation to comb production were made from different types of animal bone (horse, whale, pig, and cattle) and not antler. This was either because of difficulties in access to antler or a desire to use the raw material available within the town perimeter. A cost-effective method that would make the comb a very affordable product and probably also enable a mass production since the raw material was present in abundance. Instead of being dependant on raw material from outside the town the comb maker would operate within the town limits. Even the copper used for the rivets, that would attach the connecting plates to the tooth plates, may have been made from reused material.

These considerations also leads to the suggestion that using a specific type of antler in Viking Age combs was a very conscious and active choice since the Viking Age comb makers also would have had access to animal bones as raw material. It does not seem to have any functional or visual consequence whether animal bone or antler is used as raw material for the comb. Instead there might be some underlying symbolic meaning in the Viking Age comb makers choice of material (Ashby 2014, 99–121). The change of raw material indicates that the comb changes from being an exclusive product to a more accessible and affordable everyday product (Figure 5).

The exotica

A couple of exotic species turned up amongst the species identifications by ZooMS. One was the Atlantic walrus (Figure 6) and the other was a right whale. The piece identified as walrus was a part of walrus tusk also known as ‘the ivory of the north’ in the Middle Ages. Walrus tusk was used as gaming pieces, mounts or decorations for caskets or other decorative items. They were highly prestigious items and a luxury good. The piece from Odense may be part of a gaming piece, but it has not been possible to give a positive identification. It was found in the activity layers that had accumulated around a 14th century market stall. It is possible that it was part of what was sold from the stall but it is also possible that it was lost accidentally in the busy crowd by one of the customers.



Figure 5. A small one-piece double-sided comb made from bovid/cervid bone (x3025). Photo: Nermin Hasic, Odense City Museums.

The Atlantic walrus was traded all over Europe from Iceland and Greenland as early as the 13th century as mentioned in *Kongespejlet* (Latin: *Speculum regale* c. 1220–30 AD) (Brøgger 1947). They were hunted by the Norse who settled on the south-west coast of Greenland from around 1000 to the early 14th century (Jensen and Østergaard 2017, 178). In 1327 the Greenlandic tithes were paid through sale of walrus tusk (Liebgott 1985, 10–11; Jette Arneborg 2000, 304–305). Findings of walrus skulls in Norse farms show that it was mainly the tusks – and probably also the hide – that was the most desired part of the walrus (Arneborg 1999). Walrus found in Denmark may have been traded through Norway.

The presence of walrus tusk in Odense both evidences the trade connections of the town and also a rise in living standards paralleling trends elsewhere in Europe.

Discussion

The ZooMS analysis of bone artefacts from medieval Odense demonstrates the potential of ZooMS for species identification a group of materials that have until now remained silent: artefacts without recognisable morphological traits. Until now such



Figure 6. Two small pieces of worked walrus tusk which might have served as gaming pieces. OBM 9776 × 731. Photo: Peter Helles Eriksen.

artefacts have mainly been treated in relation to their function or decoration, but integrating ZooMS, they can also provide us with information of the choice of animal resources for bone artefacts which, as demonstrated, adds to our understanding of their production, use, and interpretation. ZooMS offers the opportunity to obtain a more complete picture of the use of animal resources by allowing us to include not only worked artefacts, but also the large material of unidentified animal bones without diagnostic traits. This not only applies to bone assemblages from medieval towns, but to animal bones across time periods, cultures, and geographical regions.

While other biomolecular methods might be used for species identification, ZooMS is cheaper and faster and more easily applicable than both regular aDNA analysis and shotgun DNA or proteomics methods. This has several advantages; for museums, this means that it is possible to perform large-scale ZooMS analysis of artefacts without over-burdening budgets. Also the species identification is within the guidelines of the Danish Museum Act.⁹ Combining traditional zooarchaeological analysis with ZooMS will enhance the outcome of both methods and give more complete data on past animal resources and how they were exploited. With the low costs it is moreover possible to do ‘bulk analysis’ that will go beyond identifying the species of different looking artefacts in the hope of detecting the presence of exotic and unexpected animals (e.g. 12,317

samples, Buckley *et al.* 2017). Instead, it is possible to ask questions that require a larger body of analysed objects and enable construction of full biographies of the practices related to the use of animal resources and reveal patterns in trade and resource networks on a global, regional, and local scale. ZooMS can even be used as a screening tool and precursor to aDNA analysis. As an example ZooMS has been applied to Palaeolithic bone assemblages to reveal archaic hominins, which could then be subjected to further biomolecular analysis that can provide higher resolution data as aDNA and shotgun proteomics (Brown *et al.* 2016, Welker *et al.* 2016). This means that museums can select for destructive analysis (e.g. isotopes, DNA, radiocarbon dating) samples which have no morphological value instead of artefacts or elements with diagnostic characteristics (e.g. the right humerus of the Neandertal type specimen, Krings *et al.* 1997).

The reliability of ZooMS as a method for species identification has been independently confirmed in several studies (e.g. Von Holstein *et al.* 2014/1; Welker *et al.* 2015b, Evans *et al.* 2016). However there are two further considerations which should be born in mind before considering undertaking a study. Firstly the rate of collagen sequence evolution is relatively slow and consequently there may be instances, such as in the case of Indian/African elephants/mammoth, in which there are no differences within type I collagen (Buckley *et al.* 2011, Coutu *et al.* 2016). This may be particularly problematic if there is a large diversity of closely related species which could be utilised (e.g. Bovidae in Africa), but ZooMS is for instance diagnostic for most domesticated animals in Scandinavia. Secondly the quality of the identification is only as good as the quality of the database against which the samples are searched. Consequently there are cases in which samples could be misidentified, if the relevant species is not available. In these cases, the best match would be a closely related species, and if no unique masses are present, the identification may be mis-called.

The success rate of ZooMS is a function of the amount of collagen present in the sample which itself will decline in older bones, will be low in bones that have been cooked and absent in bones which have been burnt. For unburnt bone, Evans *et al.* (2016) report that 35/38 archaeological whale bones were identified to order, family or genus, and Ashby *et al.* report identifications to species for antler or animal

bone for 469 of 705 combs or comb fragments from Danish Viking Age (Ashby *et al.* 2015). However, few studies report clear success rates, perhaps because samples can easily and without great cost be reprocessed. A second factor which may lead to poor results is if the extracts are highly discoloured for instance due to an abundance of humic acids from the soil. This can be a problem in urban deposits, but where this becomes a challenge is the case of tanned leather. It remains unclear as to why tanned leather has sometimes proven problematic for ZooMS (and also for DNA amplification, Vuissoz *et al.* 2007), it may be because the tanning agents interfere with the enzymes used in the assay or it may be that not all of the aromatic compounds are retained in the C₁₈ clean up step and they may interfere with the laser ionisation step. For parchment (well preserved processed animal skins) which has never been buried, the eraser method even works better than destructive sampling (Fiddymment *et al.* 2015). However as demonstrated in the case-study from Odense, above, (for which 15 of 16 of the bone samples that had been sampled destructively worked compared to one of four of the ones sampled by eraser) that in order to have a high success rate it is necessary to take a sub-sample of the bone. Although the success rate for the eraser method on worked, bone, ivory and antler, is understandably lower, the lack of a sub-sample has obvious application, not least to portable art (e.g. Coutu *et al.* 2016).

Even where destructive sampling is used, the sample size can be so small that it is still possible to do the sampling in a area where the object can be photographed or put on display in a museum without showing the sample spot.

Although this article has focused on bone, bone assemblages are by no means the only suitable material for ZooMS. Collagen is also the major protein in skin, and the potential for ZooMS has been demonstrated on parchment as well as animal skin and connective tissues (Kirby *et al.* 2013, Fiddymment *et al.* 2015). This opens up for a enormous amount of skin-based materials of archaeological or historical origin including clothing, shoes, leather goods, furnishing, containers, wrappings, book bindings and skin-based glues. The fact that the database for keratin has been expanded (Solazzo *et al.* 2013, 2017, Solazzo 2017) opens up for an array of materials based on hair, feather, nail and baleen, not to mention textiles, for which the identification of fibre is an essential question.

Leather, skin, and hair all face the same issues as bones in terms of species identification, which also for these is based on recognisable morphological traits and differences in the so-called grain pattern of skin, which varies between species, and characteristics of hair as hair diameter, the length of the fibres, and the appearance of the scales and medulla, which also varies between species. Once these morphological features are lost, all will rely upon molecular identification.

Conclusion

The analysis of the 20 samples from medieval Odense showed that there is a great and yet unexplored potential in analysing the artefacts that are made unidentifiable through manufacture. ZooMS will test assumptions on the choice of material for different purpose and enable interpretation that goes beyond the mere species identification. Through a precise and un-debatable species identification, we are able to ask questions regarding intended and unintended actions, identities, practices taking place in the town, etc. For Odense, long distance connections was demonstrated by the find of walrus tusk and the combs showed that the medieval comb maker would use the resources available to him in the town to create an affordable everyday object.

ZooMS is a fast, minimally or even non-destructive, easily applicable method for species identification, which has proven reliable and with a good success rate, and resolution for Scandinavian fauna. The potentials of ZooMS expand beyond animal bones and is for instance applied extensively for species identification of parchment (Fiddymont *et al.* 2015), but its potentials for skin and hair based materials are also great. In medieval Odense, the ongoing species identification of leather objects will enable a cross-correlation with the evidence obtained from the bone assemblage (O'Connor 2003, 3231–3235) and in Odense and in general the identifications of leather can enlighten aspects as skin trade and choices of leather for functional or signalling purposes.

At the moment ZooMS is a research method, but like many technologies before, as the approaches become standardised we are hoping that it is made available to a wider, non-specialised, audience. Like radiocarbon before it, it is hoped that in the future ZooMS will become a routine tool available to local

museums conducting contract archaeology within Danish Museums Act part 8.

Notes

1. <https://www.retsinformation.dk/Forms/R0710.aspx?id=162504> (visited 21.09.2017).
2. <http://slks.dk/fortidsminder-diger/arkaeologi-paa-land/museernes-arkaeologiske-arbejde/vejledning-om-arkaeologiske-undersoegelser/kap-6-konservering-og-naturvidenskab/#c45926> (visited 21.09.2017).
3. Diplomatarium Danicum I, I nr. 343 s. 133–34. <http://dendigitalebyport.byhistorie.dk/medieval/item.aspx?itemid=391> (accessed 09–04-2018) .
4. <http://museum.odense.dk/forskning/projekter/odenses-opstaaen> (accessed 21–09-2017).
5. A biological family of cloven-hoofed ruminant mammals including species such as bison, African buffalo, water buffalo, antelopes, sheep, goats, muskoxen, and domestic cattle.
6. A biological family of hoofed ruminant mammals including species such as elk, reindeer, fallow deer, and roe deer.
7. In theory also with horse, but this species can be eliminated by the peak 2131,1 (bovids/cervids) or 2145,1 (horse/zebra) which is often identified, but not shown in the displayed spectrum.
8. In Århus there was 2,7% horse bone in the 10th-12th century and 1,7% in the 13th –14th century. In Ribe there were 30 fragments of horse from the 8th-9th century.
9. <https://slks.dk/fortidsminder-diger/arkaeologi-paa-land/museernes-arkaeologiske-arbejde/vejledning-om-arkaeologiske-undersoegelser/kap-6-konservering-og-naturvidenskab/>.

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