INTEGRATING ZOOMS AND ZOOARCHAEOLOGY

This document summarizes the key take-aways of the discussion **combining ZooMS and zooarchaeology** from the online "Ask Us Anything" event organized the 30th of June, 2025 by Giulia Gallo, Raija Heikkilä and Pauline Raymond (Collège de France).

A **panel of six specialists** answered audience questions: Maria Codlin (University of Turin), Abigail Desmond (Harvard University), Emmanuel Discamps (University of Toulouse Jean Jaurès), Geoff Smith University of Reading), Camilla Speller (University of British Columbia) and Naihui Wang (University of Tübingen).

WHAT IS ZOOARCHAEOLOGY?

Zooarchaeology studies animal remains (e.g. bones and teeth) from archaeological sites to understand past human-animal relationships. This includes reconstructing diets, hunting practices, domestication, environmental conditions, and economic or symbolic uses of animals.

WHAT IS ZOOMS?

ZooMS (Zooarchaeology by Mass Spectrometry) is a proteomic technique that identifies animal species by analyzing collagen peptide markers preserved in bones or dentine. It is particularly useful for highly fragmented or morphologically unidentifiable remains and has become a valuable tool in both archaeological and paleoecological research.

MATERIAL SELECTION

- ZooMS can be applied not only to **fragmented bones** but also to culturally modified **artifacts**. Artifacts raise different research questions than indeterminate fragments. Researchers must ask: **What does species identification add to our understanding** (e.g., symbolic meaning, resource choice)? Is destructive sampling justified or can non-destructive methods suffice?
- **Minimally invasive sampling methods** are evolving, enabling reliable results with less destruction.
- In general, **guiding questions** can help determine if ZooMS will be useful or appropriate: Does the object lack morphological markers? What is the context (age, preservation, previous sampling success)? Are there damaged areas suitable for sampling, or important decorated surfaces to avoid?
- **Anatomically identifiable but taxonomically unclear bones** (e.g., ribs, tarsals) are good candidates for ZooMS (zooarchaeologists can set aside ambiguous bones for later analysis).
- **Bones providing seasonal or behavioral information** (e.g., fetal/neonatal bones) but no morphological features for taxonomic identification are also good candidates for ZooMS.
- Combining ZooMS with other methods (such as stable isotope analysis) can **clarify the taxonomy of ambiguous fragments**, e.g., distinguishing large tibia from reindeer vs. small red deer.

SAMPLE PREPARATION

- **Comparative specimens should be the same type of material as your samples**, because other material may contain other proteins in variable quantities.

- **Any bones can be used**, but collagen is better preserved in cortical bone. **Dentine** can also be used for ZooMS.

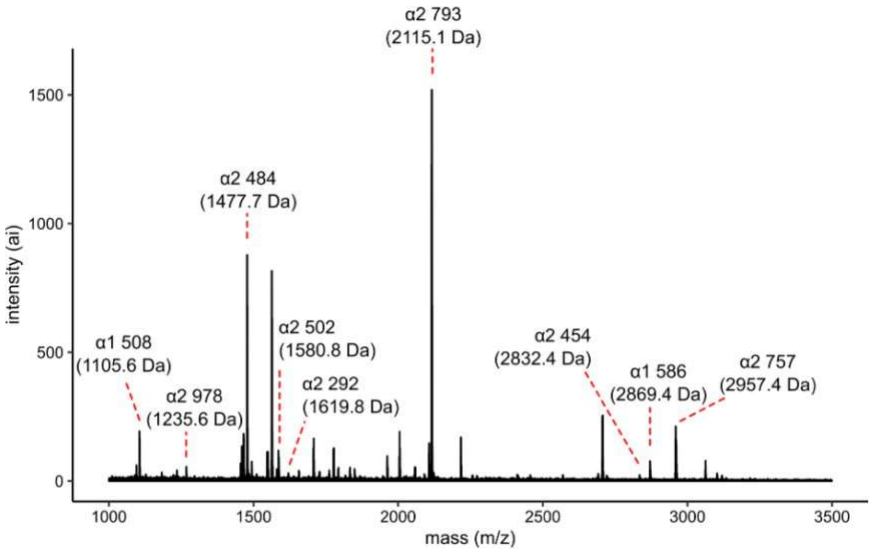
LAB EXTRACTION

- Multiple lab protocols exist to extract collagen from animal remains. The **choice of a protocol depends on the preservation and curation constraints**.
- The AmBic protocol (van Doorn et al. 2008) does not require demineralisation of the bone. The pH of this buffer solution should be between 7 and 9 for optimal trypsin activity (to digest the collagen peptides).
- Many labs **use leftover collagen from stable isotopes analyses** for ZooMS, though no formal combined workflow exists.
  Caution: High gelatinization temperatures (up to 95°C) may fragment collagen + ultrafiltration influences peptide quality.

SPECTRAL ANALYSIS

- **Manual identification** is the most common (softwares like mMass are used to read the spectra) but not well suited for large datasets.

- Emergence of **automated or semi-automated tools** such as: SpeciesScan, QuickID, PAMPA.



A complete MALDI-ToF MS spectrum from Smith et al. 2024

- **No universal peak picking setting** for ZooMS, it depends on: sample quality, instrument performance and background noise.
  Critical points:
- **Signal-to-noise ratio (S/N):** important to adjust depending on the instrument.
- **Isotopic cluster shape** for peak reliability: triangular shape for low masses (m/z <2500) and bell/normal distribution for high masses (m/z >2500).
- Overabundance of peaks often results from pre-processed spectra (e.g., saved as .msd files with prior baseline correction). Best practice: start from **raw data to ensure accurate peak detection**.
- The tallest peak is not always the correct mass, especially at higher m/z or in low-quality samples.
- You can **merge spectra** from multiple MALDI for a single sample or analyse individual spectra (recommended for low-quality samples).
- **Not all markers are equally diagnostic**: Marker P1 is common across mammals, Marker A is highly distinctive for reindeer, Marker E is can be missing due to mis-cleavage and poor ionisation.
- Demineralization and repeated digestions can help **differentiate contaminant from original bone**. If host and contaminant are closely related (e.g., cow glue on auroch bone), separation is nearly impossible. Museum records are valuable but often incomplete.
- **Bird** identification is difficult due to overlapping peptides and limited reference databases. Instead of spreadsheet formats, reorganizing data to show all possible peptides and manual checking is advised.

ZOOMS REFERENCE DATABASES

- Building reliable datasets involves combining recent natural history collections (to extract collagen of taxidermized animals) and archaeological references, especially for extinct or locally altered fauna.
- University of York hosts an **international ZooMS marker database** accessible to all.
- Specialized publications can provide additional family- or genus-level markers
- Databases are still being developed outside of Europe/North America to fill in the current gaps.
- Currently, sex determination is done using shotgun proteomics via LC-MS/MS. Adapting to ZooMS would require a specialized database and enhancing low-abundance Y peptides.

INTEGRATING ZOOMS AND ZOOARCHAEOLOGY: APPLICATIONS AND CHALLENGES

- Bones selected for ZooMS are not automatically included in zooarchaeological or taphonomic analysis because they can bias results (e.g. frequency of cut marks), especially if many are very small and under the cut-off size of the analyses on the identifiable portion of the assemblage.
- Basic zooarchaeological data such as bone type, element, length, or weight may be recorded to support future analyses. Detailed taphonomic information is not always necessary unless it contributes to the research question or notable features like cut marks are present, in which case those should be documented. **Partial taphonomic information from small ZooMS fragments** can still help understand site formation processes and dietary patterns.
- When ZooMS results include spatial coordinates, they enable finer-scale analysis of prey shifts and assemblage distinctions beyond what morphology alone reveals, especially with small sample sizes.
- **Taxonomic labels**: ZooMS specialists and zooarchaeologists may use different taxonomic labels. Good communication about the context (regional fauna, timescale) are necessary for better taxonomic identifications report. Don't panic if you receive an unexpected species identification!
  → Clear reporting standards and better communication between ZooMS specialists and zooarchaeologists improve interpretations.
- **ZooMS often identifies only to family level**, further species-level interpretation requires further context (i.e. data from the identifiable faunal assemblage or archaeological details such as environment and time period).
- **Fragmentation biases** affect taxonomic representation: some species may appear more abundant in ZooMS due to bone fragmentation patterns. Comparing ZooMS with morphologically identified animal remains helps assess biases.

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You can watch the recording of the online meeting on our YouTube channel:

https://www.youtube.com/watch?v=loAgeUuK08g