

PROGRAMME BOOKLET

Integrating ZooMS and zooarchaeology: methodological challenges and interpretive potentials

WORKSHOP

**18-19th April 2023,
University of Kent,
Canterbury, UK**

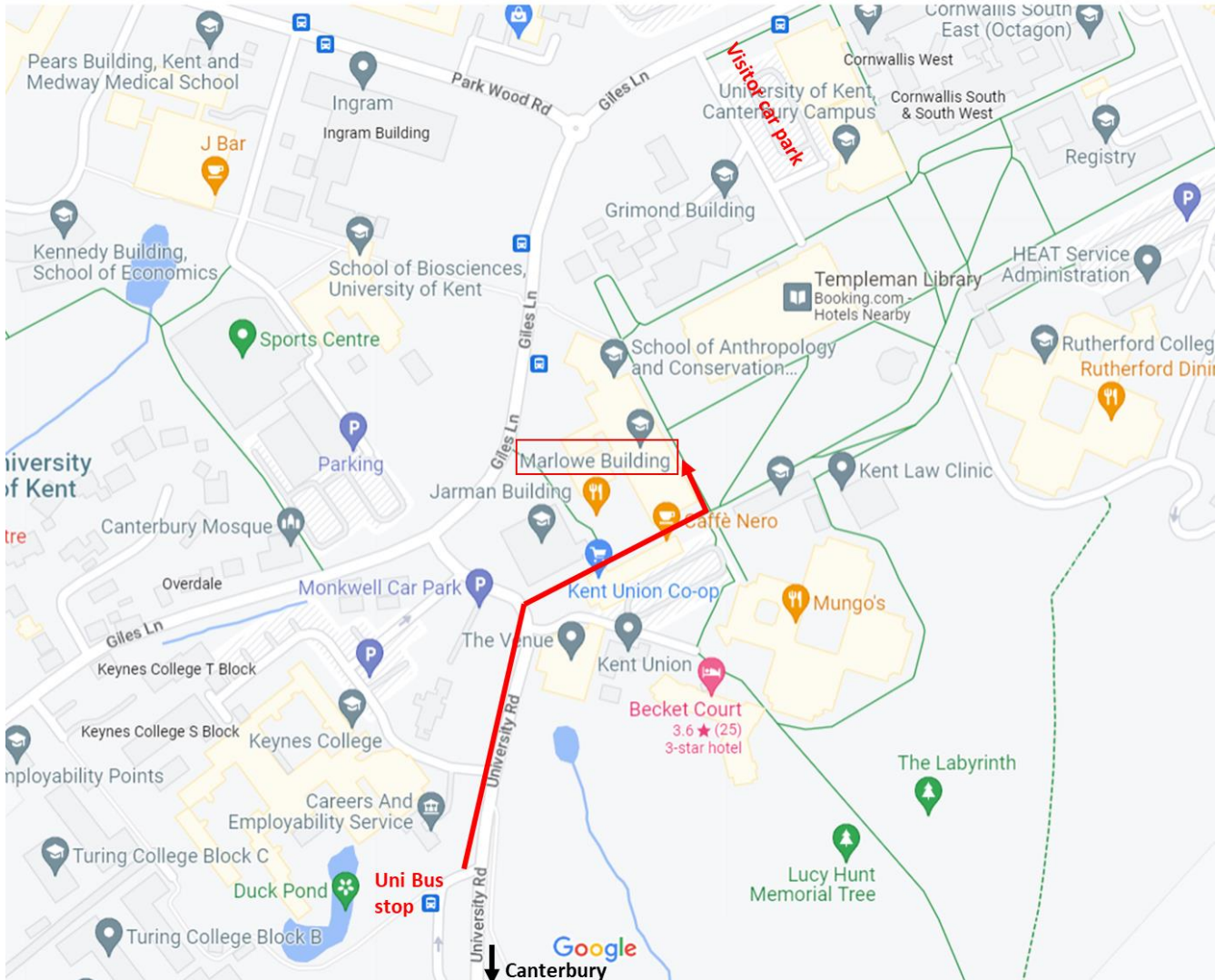
Organisers:
Dr. Geoff M Smith
Dr. Karen Ruebens
Dr. Virginie Sinet-Mathiot
Dr Frido Welker

Marie Skłodowska-
Curie Actions



University of
Kent

The workshop is being held in the **Marlowe building**,
University of Kent, Canterbury, CT2 7NR.



The keynote talks on Monday April 17th take place in **Marlowe Lecture Theatre 1**
(on the ground floor).

The workshop itself is being held in the **Swingland Room**.

How to get to the Swingland Room:

Enter Marlowe via the doors at the north end and go upstairs. Turn right and along the corridor, at the end of the corridor turn right and the room is on your right.

PROGRAMME

MONDAY APRIL 17

4:15 PM	Registration
4:30 PM	G.M. Smith (University of Kent, UK)
	The ecology, subsistence and diet of 45,000-year-old <i>Homo sapiens</i> at Bacho Kiro Cave (Bulgaria): an integration of zooarchaeology and biomolecular datasets.
5:00 PM	F. Welker (University of Copenhagen, Denmark)
	Proteomic approaches to Middle and Late Pleistocene Europe.

TUESDAY APRIL 18

9:15 AM	Registration
9:30 AM	Welcome
9:35 AM	A. B. Marín-Arroyo (University of Cantabria, Spain) <i>Chair: Geoff Smith</i>
	New biomolecular approaches applied to archaeozoology to unravel human subsistence strategies during Iberian Prehistory.
9:55 AM	L. Michaelis (University of Tübingen, Germany)
	Exploring taphonomic and selection pressures at Geißenklösterle Cave (Swabian Jura, Southwestern Germany) using ZooMS.
10:15 AM	V. Sinet-Mathiot (Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany)
	Integrating the morphologically unidentifiable fauna using ZooMS to complement our understanding of hominin subsistence strategies during the Middle to Upper Palaeolithic transition.
	Coffee break
11:00 AM	K. Ruebens (Collège de France, Paris) <i>Chair: Frido Welker</i>
	A multifaceted study of morphologically unidentifiable bone from the open-air Neanderthal site of Salzgitter-Lebenstedt (Germany).
11:20 AM	G. Pothier-Bouchard (University of Montreal, Canada)
	ZooMS-informed archaeozoological analysis comparing Neanderthal and <i>Homo sapiens</i> subsistence behaviours at Riparo Bombrini (Liguria, Italy).
11:40 AM	P. Raymond (Collège de France, Paris)
	Identifying Early Aurignacian bone fragments from Le Piage (Lot, France) using MALDI-TOF and MALDI-FT-ICR mass spectrometry.
12:00 PM	F. Holloran (University College London, UK)
	ZooMS, Zooarchaeology and Environmental reconstruction research at UCL Institute of Archaeology.
	Lunch
1:30 PM	A. Oertle (University of Vienna, Austria) <i>Chair: Virginie Sinet-Mathiot</i>
	Ancient collagen preservation in New Guinea sites: Testing applicability of ZooMS in tropical settings
1:50 PM	N. Wang (Max Planck Institute of Geoanthropology, Jena, Germany)
	The zooarchaeological research history at Vogelherd Cave: comparing zooarchaeological and ZooMS results
2:10 PM	W. Rendu (CNRS)
	From the Atlantic shore to the Central Asian Mountain : ZooMS as a key for Zooarchaeologists
2:30 PM	T. Tsutaya (University of Copenhagen)
	The potential of ZooMS to further develop stable isotope-based zooarchaeological studies

	Coffee break
3:15 PM	C. Peters (Max Planck Institute of Geoanthropology, Jena, Germany) <i>Chair: Karen Ruebens</i> Integrating ZooMS and zooarchaeology at the Late Pleistocene site of Devil's Lair (Southwest Australia)
3:35 PM	L. Torres-Iglesias (University of Cantabria, Spain) Integration of ZooMS and Zooarchaeology to assess human subsistence behaviour and zooarchaeological methodology
3:55 PM	E. Discamps (CNRS UMR5608 TRACES, Toulouse, France) Integrating ZooMS data in the reconstruction of Palaeolithic subsistence strategies and post-excavation stratigraphies: what's the point of view of a "traditional" zooarchaeologist?
4:15 PM	Tour of the School of Anthropology and Conservation, incl. Imaging Centre for the Life Sciences
5:30 PM	Workshop dinner: The Foundry Brew Pub (77 Stour St., Canterbury CT1 2NR)
WEDNESDAY APRIL 19	
9:30 AM	Welcome
9:35 AM	M. Saunders (University of Tuebingen, Germany) <i>Chair: Geoff Smith</i> Counting Fragments: A New Holistic Approach to Quantifying ZooMS-Identified Bone Fragments for Analysis
9:55 AM	I. Rodriguez Palomo (University of Cambridge, UK) High-throughput analysis and batch effect in ZooMS MALDI-TOF data
10:15 AM	E. Vegh (University of Vienna, Austria) SpecieScan: Automated Species Identification of Bone Fragments
	Coffee break
11:00 AM	Discussion session part 1: Optimising ZooMS data for zooarchaeological studies We will discuss all aspects of the ZooMS protocol from sampling strategies, extraction methods and data processing to species identification and biomolecular preservation.
12:30 PM	Lunch
1:30 PM	Discussion session part 2: Optimising ZooMS data for zooarchaeological studies We will discuss ways forward to come to a fuller integration of ZooMS and zooarchaeological datasets, including the recording of zooarchaeological attributes, data reporting, quantification indices and data availability.
3:00 PM	Closing comments
3:30 PM	final coffee

KEYNOTE TALKS**Monday April 17th 4:30-5:30 PM****Dr Geoff M. Smith****The ecology, subsistence and diet of 45,000-year-old *Homo sapiens* at Bacho Kiro Cave (Bulgaria): an integration of zooarchaeology and biomolecular datasets***School of Anthropology and Conservation, University of Kent, UK.*

Zooarchaeology is a powerful tool to understand how hominin groups adapted to changes in climate and environment through examination of their subsistence and dietary behaviours. These reconstructions are reliant on the correct morphological identification of bone fragments, though this is often complicated by the high degree of bone fragmentation in Pleistocene bone assemblages. The application of novel biomolecular datasets (ZooMS, stable isotopes, ancient DNA) with zooarchaeological data has provided a new wealth of opportunities to investigate human behavioural adaptation. This talk will use the recently excavated and analysed bone remains from Bacho Kiro Cave (Bulgaria) to illustrate how the combination of such approaches provides fresh perspectives on late Neanderthals and early *Homo sapiens* behaviour.

**Dr Frido Welker****Proteomic approaches to Middle and Late Pleistocene Europe***Section for Molecular Ecology and Evolution, Globe Institute, Copenhagen, Denmark.*

Departing from the observation that ZooMS has potential contributions to make to zooarchaeological studies, here I will focus on the possible further insights to be gained from palaeoproteomic methods in the study of Middle and Late Pleistocene hominins, including their behaviour and phylogenetic relationships. To enable such analyses to fulfill their potential, we first require to reconsider some of the more practical, methodological aspects of palaeoproteomics studies generally. For example, limitations exist in the taxonomic assignments made through ZooMS, which can be further resolved using shotgun proteomic approaches. In turn, these shotgun proteomic methods should enable the recovery of skeletal proteomes informative on additional aspects of the life and death of archaic hominins.

ABSTRACTS

New biomolecular approaches applied to archaeozoology to unravel human subsistence strategies during Iberian Prehistory

Ana B. Marín-Arroyo¹, Leire Torres-Iglesias¹, Elene Arenas Sorriqueta¹, Lucia AgudoPérez¹, Fei Yang¹, Joseba Rios², Laura Sanchez-Romero³ and Frido Welker⁴.

¹*Grupo I+D+i EvoAdapta, Dpto. de Ciencias Históricas, Universidad de Cantabria, Santander, Spain.*

²*Arkeologi Museoa, Bilbao, Spain, Centro Nacional de Investigación sobre la Evolución Humana (CENIEH), Burgos, Spain*

³*Autonomous University of Barcelona, Spain.*

⁴*Globe Institute, University of Copenhagen, Copenhagen, Denmark.*

Today, there is a dialogue between archaeozoology and biomolecular archaeology which is providing fruitful insights into human subsistence strategies during Prehistory.

When studying an archaeomammal assemblage, ZooMs has become recently a relevant tool to enlarge our knowledge of taxonomy, especially in those highly fragmented Paleolithic contexts, when usually less than 10% of the skeletal remains are identified into taxa. However, this method must not be considered a panacea but a supportive methodological approach for archaeozoologists. The research questions and type of samples to be analysed must be clear before applying the method.

We want to focus this presentation on dealing with 1) the possible research questions that we need to consider and can propose when ZooMs methodology wants to be applied; 2) how we should design a reasonable sampling strategy; 3) how the quantification of the assemblage needs to be evaluated and finally, 4) how we can evaluate the combined molecular and archaeozoological results.

We present several case studies applied to different Paleolithic and Mesolithic sites in northern Iberia.

Exploring taphonomic and selection pressures at Geißenklösterle Cave (Swabian Jura, Southwestern Germany) using ZooMS

Luca E. Michaelis¹, Susanne C. Münzel¹, Keiko Kitagawa^{2,3}, Britt M. Starkovich^{1,2}, Nicholas J. Conard^{2,3}, Samantha Brown¹

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³Department for Early Prehistory and Quaternary Ecology, University of Tübingen, Tübingen, Germany.

Studying osseous material within archaeological assemblages is often affected by taphonomic and anthropogenic factors which hamper morphological approaches to taxonomic identification. To address this, we carried out a combined zooarchaeological and ZooMS study of a little over 1000 unidentifiable bones from Middle Paleolithic (MP), Aurignacian and Gravettian contexts at Geißenklösterle Cave in the Ach Valley of the Swabian Jura, Southwestern Germany. The cave is significant to the study of the Paleolithic in Europe as it covers the Middle to Upper Paleolithic transition and contains some of the earliest evidence for the Aurignacian so far found in Central Europe. The results reveal a high level of biomolecular preservation within Geißenklösterle Cave with ~96% of the samples containing sufficient collagen to be taxonomically identified. While the emergent data mostly agrees with the morphological studies regarding species composition, there were notable findings, including the first musk ox (*Ovibos moschatus*) identified in stratigraphic association with the Aurignacian in Central Europe and a cut-marked bone of a woolly rhinoceros (*Coelodonta antiquitatis*) specimen from the MP, the first of its kind for the Swabian Jura.

Taking all this into account our data shows the potential of gaining a more holistic insight into the ecological behavior of the Neanderthals and modern humans that once occupied the Ach Valley when combining morphological and biomolecular methods of bone identification, and is extremely promising regarding further in-depth studies of the faunal material found within the cave sites of the Swabian Jura.

Integrating the morphologically unidentifiable fauna using ZooMS to complement our understanding of hominin subsistence strategies during the Middle to Upper Palaeolithic transition.

Virginie Sinet-Mathiot¹, William Rendu², Teresa E. Steele³, Rosen Spasov⁴, Stéphane Madelaine^{5,6}, Sylvain Renou⁷, Marie-Cécile Soulier⁸, Naomi L. Martisius⁹, Vera Aldeias¹⁰, Elena Endarova⁴, Paul Goldberg^{10,11,12}, Shannon J.P. McPherron¹, Zeljko Rezek¹³, Dennis Sandgathe¹⁴, Nikolay Sirakov¹⁵, Svoboda Sirakova¹⁵, Marie Soressi¹⁶, Tsenka Tsanova¹⁷, Alain Turq⁵, Jean-Jacques Hublin^{13,1}, Frido Welker¹⁸, Geoff M. Smith¹⁹

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4 - Archaeology Department, New Bulgarian University, Sofia, Bulgaria.

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8 - CNRS UMR 5608 TRACES, Université de Toulouse-Jean Jaurès, Maison de la Recherche, Toulouse, France.

9 - Department of Anthropology, The University of Tulsa, Tulsa, OK, USA

10 - Interdisciplinary Center for Archaeology and the Evolution of Human Behaviour, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal

11 - School of Earth, Atmospheric and Life Sciences, University of Wollongong, Australia

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13 - Chaire de Paléanthropologie, CIRB (UMR 7241 – U1050), Collège de France, Paris, France.

14 - Department of Archaeology, Simon Fraser University, Burnaby, British Columbia, Canada.

15 - National Institute of Archaeology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria.

16 - Faculty of Archaeology, Leiden University, The Netherlands.

17 - Department of Chemistry G. Ciamician, Alma Mater Studiorum, University of Bologna, Italy

18 - Globe institute, University of Copenhagen, Copenhagen, Denmark.

19 - School of Anthropology and Conservation, University of Kent, Canterbury, UK.

The comprehensive taxonomic identification of faunal remains is crucial to understanding Palaeolithic hominin subsistence strategies. Due to high fragmentation, bone morphology is often insufficient to taxonomically identify Late Pleistocene faunal assemblages. In this study, we address the implications of integrating morphologically unidentifiable components into the general understanding of the formation of three faunal datasets from Bacho Kiro Cave (Bulgaria), Les Cottés and La Ferrassie (France), and of subsistence strategies during the Middle to Upper Palaeolithic transition (MUPT). Our results show an under-representation of the proportion of the large ungulates, e.g., Bos/Bison and equids, at Les Cottés and La Ferrassie across the MUPT. The zooarchaeological analysis of these bones highlights a potential differential identification rate between taxa, notably towards reindeer. This, possibly creates a reporting bias in the representation of the dominant taxa depending on their ease of identification. The assessment of the fragmented component of the bone material from Les Cottés refines shifts in taxonomic proportions of large ungulates across the MUPT with the progressive decreased abundance of Bos/Bison, counterbalanced by a progressive increase of an underestimated exploitation of Equidae. In addition, the incidence of carnivores could be assessed confirming a progressive decrease of carnivore activity alongside the expansion of early modern humans at Bacho Kiro Cave and Les Cottés. By combining traditional and biomolecular methods, (zoo)archaeologists can overcome some of the methodological limitations commonly encountered during morphological assessments of Palaeolithic bone assemblages, especially at noteworthy periods in human history (e.g., MUPT).

A multifaceted study of morphologically unidentifiable bone from the open-air Neanderthal site of Salzgitter-Lebenstedt (Germany)

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Pleistocene faunal assemblages are often highly fragmented, hindering taxonomic identifications and interpretive potentials. In this paper, we apply four different methodologies to morphologically unidentifiable bone fragments from the Neanderthal open-air site of Salzgitter-Lebenstedt (Germany). First, we recorded zooarchaeological attributes for all 1,362 unidentifiable bones recovered in 1977. Secondly, we applied Zooarchaeology by Mass Spectrometry (ZooMS) to 761 fragments, and calculated glutamine deamidation values. Thirdly, we assessed the collagen preservation of 30 fragments by near-infrared spectroscopy (NIR) and, finally, we pretreated ten bones with high predicted collagen values for radiocarbon dating. All returned dates at or beyond the limit of radiocarbon dating, indicating an age of older than 51,000 years ago. The ZooMS faunal spectrum confirms a cold environment, dominated by reindeer, alongside mammoth, horse and bison. The low occurrence of carnivore modifications (1%) contrasts with an abundance of human modifications (23%). Cut marks and marrow fractures were observed across reindeer, horse and bison. The mammoth remains are less well-preserved and show a lower degree of human modifications, indicating, perhaps, a different taphonomic history. Overall, this study illustrates the importance of retaining, studying and incorporating the unidentifiable bone fraction to optimize interpretations of site formation and subsistence behaviour at Palaeolithic sites.

ZooMS-informed archaeozoological analysis comparing Neanderthal and *Homo sapiens* subsistence behaviours at Riparo Bombrini (Liguria, Italy)

Geneviève Pothier-Bouchard^{1,2}, Ariane Burke², Fabio Negrino³, Michael Buckley⁴, and Julien Riel-Salvatore²

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⁴ *Manchester Institute of Biotechnology, University of Manchester*

Located in the Balzi Rossi Paleolithic site complex, Riparo Bombrini documents one of the latest Mousterian occupations in Liguria (Italy), along with early Protoaurignacian occupations. Previous studies on the late Mousterian (MS) and Protoaurignacian (A1, A2) levels have revealed distinct mobility and technological organizations associated with changing paleoenvironment signatures. This makes Bombrini a key site to document the subsistence adaptations and human-environment interactions of the last Neanderthals and the subsequent arrival of modern humans in Western Europe.

We present the analysis of levels MS, A1, and A2 using an integrated methodological approach that combines archaeozoological methods, multivariate taphonomic analysis and FTIR-aided mass ZooMS sampling. The results provide the first high-resolution study of human subsistence during the Middle-Upper Paleolithic transition (43-36 ky cal BP) in the Liguro-Provençal arc region. These data allow us to test for the possible existence of inter-population differences in behaviour and adaptation in the context of rapid climate change on the European continent.

Identifying Early Aurignacian bone fragments from Le Piage (Lot, France) using MALDI-TOF and MALDI-FT-ICR mass spectrometry.

Pauline Raymond¹, Karen Ruebens¹, Fabrice Bray², Jean-Christophe Castel³, Eugène Morin⁴, Foni le Brun-Ricalens⁵, Christian Rolando², Jean-Guillaume Bordes⁶, Jean-Jacques Hublin¹

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Since the development of paleoproteomic studies, ZooMS (Zooarchaeology by Mass Spectrometry)¹ has been applied to different Paleolithic sites to identify the morphologically unidentified bone remains. Here we report new ZooMS analyses at the site of Le Piage (Lot, France) on 1045 bone fragments. Moreover, we used a subset to test different ZooMS protocols, including various extraction methods and different mass spectrometers. Since 2004 the excavations at Le Piage are led every year by Jean-Guillaume Bordes². The site displays a high density of archaeological material and, all the bone fragments included in this study come from a single square meter (I5C). We focused on an Early Aurignacian layer ($32\,480 \pm 300$ BP), a period critical to understanding early *Homo sapiens* settlements in Western Europe. Bone fragments were retrieved through sieving (1.5 - 4 cm) and were too highly fragmented to allow a morphological taxonomical determination.

ZooMS is based on the study of collagen, which is the most abundant protein in bones, and can discriminate different animal taxa, through peptide mass fingerprinting. Because of the variable collagen preservation of the bones comprised in this study and the development of new techniques in paleoproteomics, 87 bones were selected for a methodological comparison. Three protocols of collagen extraction were tested^{1,3} and two different mass spectrometers (MALDI-ToF and MALDI-FT-ICR) were used.

Overall, ca. 70% of the bone fragments could be identified. The spectrum of identified species by ZooMS differs to that established by the morphological analysis of the best-preserved bone material. While reindeers are consistently dominant, bovids and other cervids are more abundant in the ZooMS spectrum. A rare taxon on the site, the hare, was also identified using ZooMS. The methodological comparison demonstrates firstly that the lab protocol has to be chosen according to the mass spectrometer used to obtain the best results. Secondly MALDI-FT-ICR is a useful tool that can be used complementary to MALDI-ToF analysis to answer specific research questions (e.g. identify taphonomically altered bone).

¹ Buckley M. et al. (2009) Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry* 23, 3843-3854.

² Border J-G. et al. (2009) Les débuts du Paléolithique supérieur dans le Sud-Ouest de la France: Nouvelles fouilles au Piage (2004-2006). In «Les sociétés Paléolithiques d'un grand Sud-Ouest : nouveaux gisements, nouvelles méthodes, nouveaux résultats», *Bulletin de la Société Préhistorique française*.

³ van Doorn N.L. et al. (2012) – Site-specific deamidation of glutamine: a new marker of bone collagen deterioration, *Rapid Communications in Mass Spectrometry* 26, 2319-2327.

ZooMs, Zooarchaeology and Environmental reconstruction research at UCL Institute of Archaeology.

Fiona Holloran, Delphine Frémondeau, Louise Martin and Rhiannon E. Stevens

Institute of Archaeology, University College London, UK

The development of biomolecular techniques to extract data from morphologically unidentifiable bone specimens facilitates deeper questioning of an abundant but often discarded resource. A range of research projects at UCL Institute of Archaeology include the extraction of data from such indeterminate bone fragments. These projects are applying zooarchaeological analysis to determine bone fragmentation patterns and taphonomic history, peptide mass fingerprinting using ZooMS (Zooarchaeology by Mass Spectrometry) to identify the species present, and stable isotope analysis to infer climate and the food web. In combination, these techniques can open a broad window into the human past.

Our presentation will introduce a selection of research projects being undertaken at UCL, with a particular focus on Middle and Upper Palaeolithic sites in Britain. At this time, Britain was a distant outpost of human occupation in Europe with more varied temperatures and small human groups coming and going. As such, it offers an opportunity to gain further insight into the ecological plasticity of humans and their cultural ability at the furthest extent of their geographical range. By targeting bone fragments from the same archaeological context as lithics, insight into the environment that these human groups may have experienced can be inferred.

Ancient collagen preservation in New Guinea sites: Testing applicability of ZooMS in tropical settings

Annette Oertle, Naihui Wang, Glenn Summerhayes, Sue O'Connor, Matthew Spriggs, Mary-Jane Mountain, Tim Denham, Katerina Douka

University of Vienna, Austria.

The Pleistocene and early Holocene archaeological and, especially, palaeoanthropological records of the New Guinea region are sparse and poorly understood. Preservation issues in tropical environments play a substantial role in this and as such, the rarity of hominin fossils from this region is a significant limitation in testing current hypotheses on Denisovan presence in Sahul during the Late Pleistocene. Through the extensive application of Zooarchaeology by Mass Spectrometry (ZooMS) analyses of unidentified bone fragments, new hominin fossils can be identified if ancient collagen is preserved.

This paper presents ZooMS results stemming from FINDER Project (ERC-715069) with 535 samples from 10 New Guinea sites; an attempt that has succeeded in expanding the record of hominin fossils in this region. We also observed improved preservation of archaeological collagen in the New Guinea Highlands compared to the coastal regions. These analyses provide the perfect opportunity to test organic material preservation in archaeological bones to find candidate samples not only suitable for palaeoproteomic analyses, but also for isotopic, dating, and genetic applications. Additionally, the widespread screening of old collections and material excavated, even several decades ago, has the potential to provide significant new information for the prehistoric record of SE Asia and Oceania.

The zooarchaeological research history at Vogelherd Cave: comparing zooarchaeological and ZooMS results

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⁵Research Laboratory for Archaeology and the History of Art, University of Oxford, Dyson Perrins Building, South Parks Road, Oxford OX1 3QY, United Kingdom

The analysis of archaeological bones, which is broadly defined as zooarchaeology, has been since its inception a truly interdisciplinary field of research, also informing other applied methods such as radiocarbon dating, stable isotope and ancient DNA analysis. Yet, the development and broader application of palaeoproteomics, and especially ZooMS, is making new impetus to zooarchaeology.

In this paper, we use newly obtained data from Vogelherd Cave, a prehistoric site in south-western Germany, to show how ZooMS can accompany traditional zooarchaeological approaches.

Vogelherd Cave is known for the earliest evidence of figurative art in the Aurignacian around 43-35,000 years ago. The cave was initially excavated in 1931 when nearly 500 m³ were dug out in just three months. Between 2005 to 2012, the University of Tübingen revisited the site and excavated the backfill. Hundreds of Aurignacian ivory beads were identified from water screening sediments.

The faunal assemblages of Vogelherd have been studied at different periods: first, in 1954, palaeontologist Lehmann published a report on a portion of the diagnostic elements, while in the early 2000s, Niven re-examined all the specimens of the first excavation in the most comprehensive zooarchaeological study of Vogelherd so far. Later, in 2014, Boger studied the fauna remains recovered from the backfill.

Here, we present results of the first ZooMS application on the fragmentary bones (n=297) from the backfill of the cave, including the oldest human fossil found at Vogelherd. By comping the taxonomic datasets (zooarchaeology and ZooMS), we will the advantage of integrating the two approaches and attempt to build a comprehensive profile of the site's fauna, as well as the history of human occupation.

From the Atlantic shore to the Central Asian Mountain: ZooMS as a key for Zooarchaeologists

William Rendu¹, Svetlana Shnaider², Malvina Baumann³ et al

¹CNRS

²*Institute of Archaeology and Ethnography of the Siberian Branch of the Russian Academy of Sciences*

³*Traceolab, Liege University*

Zooarchaeologists rely primarily on the identification of the dental and skeletal remains found in archaeological context and their potential anthropic or natural modifications. The identification process is always the same, recognition of the anatomical portion and then the species. However, when the material is too fragmented or numerous taxa of same size are present in faunal spectra, it is not always possible to obtain the taxonomic identification. In the same way, some assemblages are characterized by a limited frequency of carnivore or human modifications; considering that in most zooarchaeological analyses the percentage of identification is lower than 10% it is crucial to obtain the taxonomic ID of the rare fragments with marks in order to precise our understanding of past human behavior.

Thus, as zooarchaeologist and ZooMS specialists, we have teamed up together to tackle this issue of taxonomic identification in the different contexts we are working on. Through two specific examples, this presentation aims to show how the integration of ZooMS in classical zooarchaeological analyses can push forward our understanding of past human and animal societies.

The French Middle Paleolithic site of Chez Pinaud has yielded an important bone industry mostly produced on large ungulates remains, sometimes heavily modified limiting their identification and preventing us to discuss the potential selection made by the Neanderthal for their production.

The site of Istiskaiya is a cave at more than 4000 meter above sea level in the Pamir Mountain of Tadjikistan. While the zooarchaeological analysis recorded several human modifications on the remains, their intense taphonomic fragmentation considerably limited their taxonomic attribution.

In these two cases ZooMS was the key to unlock our perception of the human activities.

The potential of ZooMS to further develop stable isotope-based zooarchaeological studies

Takumi Tsutaya

The Graduate University for Advanced Studies, University of Copenhagen

Integration of different methods in archaeological science can illuminate the detailed picture of animal behaviors in the past, palaeoenvironmental changes, and their interactions. In this study, diachronic changes in human fishing practices and marine ecosystems were investigated for Epi-Jomon (299–258 BC) and Okhotsk (489–1200 AD) periods in prehistoric Hokkaido, northern Japan, by utilizing the quantitative zooarchaeological method and stable isotope analysis. Carbon and nitrogen stable isotope ratios of bulk collagen of 242 fish bone samples, representing 12 taxa, excavated from the site of Hamanaka 2 on Rebun Island revealed significantly lower ($p < 0.05$) nitrogen isotope ratios in cod from the Okhotsk period than the Epi-Jomon period. Also, the vertebra diameter was significantly smaller in cod from the Okhotsk period. These differences could be related to the development of fishing gear and/or to changes in fishing strategies in the Okhotsk period, as well as to changes in the behavior of cod because of the rapid cooling climate event separating the two periods. However, due to the low taxonomic resolution of morphological species identification for fish vertebra, there is room for the improvement of reconstruction of diachronic changes in human behaviors and the marine environment. ZooMS will be discussed as the promising method to improve such isotope-based zooarchaeological studies.

Integrating ZooMS and zooarchaeology at the Late Pleistocene site of Devil's Lair (Southwest Australia)

Carli Peters¹, Noel Amano¹, Joe Dortch², Nicole Boivin^{1,3,4}

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Devil's Lair is an important archaeological site in Southwest Australia that currently provides the earliest evidence for human occupation in the region at around 50 thousand years ago. The site thus holds significant potential to provide us with critical information to better understand early human exploitation of local endemic fauna in this region. However, the faunal assemblage at Devil's Lair is highly fragmented by the combined effects of human and carnivore activities. This has, to date, significantly limited the number of bones that can be identified morphologically at the site. This study makes use of Zooarchaeology by Mass Spectrometry (ZooMS) to identify highly fragmented bones from the earliest occupation phases of Devil's Lair (~30-50 kyr) to help to partially overcome these issues and complement previous zooarchaeological work at the site. Here, we present the preliminary ZooMS results of analyzed bone fragments from Devil's Lair. The ZooMS data is compared with and incorporated into existing zooarchaeological and bulk bone DNA metabarcoding datasets to acquire a more in-depth understanding of the faunal assemblage at Devil's Lair. We also explore what we can learn from the different datasets and how they can be best combined for maximum effect.

Integration of ZooMS and Zooarchaeology to assess human subsistence behaviour and zooarchaeological methodology

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The high fragmentation of Palaeolithic faunal assemblages is a problem in zooarchaeological studies as it prevents a precise taxonomic identification following morphological criteria. Zooarchaeology has solved this issue by attributing non-identifiable bone remains to a series of mammal size classes according to mainly bone cortical thickness. In this study, we combine ZooMS and zooarchaeological and taphonomic data to infer the subsistence strategies of Solutrean and Magdalenian human groups of La Viña rock shelter (northern Iberia), as well as to assess the success rate of the mammal body size classification. The prey ranking obtained from the combined results is dominated by cervids, mainly red deer, and complemented by caprids, horse and large bovids. ZooMS results are consistent with the morphological identifications and identify horse as the main species included in the large mammal size, whereas large bovids are a minor component. The combination of ZooMS results and mammal size data shows that this classification was successful in most cases. However, it is important to consider the high variability of red deer, as their remains were classified in all body size classes. The cortical thickness dimensions of long bones show a wide range in some species such as horse and red deer. This overlap may be due to possible body overlapping between individuals of different age and sex, skeletal variation of this measurement or even fracture angle. Therefore, it is important to take these data with caution as they may not fully reflect the actual taxonomic composition of the studied assemblages.

Integrating ZooMS data in the reconstruction of Palaeolithic subsistence strategies and post-excavation stratigraphies: what's the point of view of a "traditional" zooarchaeologist?

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ZooMS has seen considerable development and increased use in the last decade. Most of the time, it has been applied by proteomic specialists, highlighting the potential of the method, but yet with little integration of the data produced in the general framework of zooarchaeological interpretations. The actual impact of ZooMS identifications on our understanding of past subsistence strategies, prey selection, transport decisions or butchering activities has yet to be fully uncovered. Taking a step back, we here frame theoretical considerations on the limits and benefits of ZooMS identifications, from the point of view of a "traditional" zooarchaeologist trained in the morphological identification of bone fragments in Paleolithic contexts, in charge of the analysis of large faunal assemblages. Issues such as bone fragmentation and differential identification (according to skeletal element and/or species) might have a dramatic impact on abundance measurements derived from ZooMS data. After highlighting the specificities of these biases, we explore ways of counterbalancing them using numbers of distinct elements, bone measurements and weights, and test our hypotheses using ZooMS data recently acquired on Middle and Early Upper Palaeolithic assemblages from southwestern France. Finally, through practical examples, we call attention to an additional key interest of ZooMS when it is applied to unidentified plotted bones: the definition of Post-Excavation Stratigraphies (cf. <https://doi.org/10.21203/rs.3.rs-2405909/v1>), and the increased reliability and resolution that ZooMS data can offer in our understanding of faunal changes when interpreted spatially.

Counting Fragments: A New Holistic Approach to Quantifying ZooMS-Identified Bone Fragments for Analysis

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The application of ZooMS to Pleistocene-aged osseous assemblages has allowed for novel insights into human evolution and behavior. Recent ZooMS research suggests that there may be significant data “hidden” within the fragmentary portions of faunal assemblages which traditional zooarchaeological methods are unable to access. One of the most significant concerns with the integration of ZooMS identifications, however, is that the quantification of these fragments is currently restricted to NISP counts, which bring up obvious issues with double-counting that standard zooarchaeological quantification methods (e.g., MNI, MNE, DZ, etc.) have worked to excise. This project has therefore sought to develop a new weight-based statistical method for quantifying ZooMS-identified fragments, abstracted from Kubasiewicz’s Wiegemethode, which the authors have termed Relative Abundance (RA). Since RA incorporates fragmentation rates, body part representation, and total expected skeletal weight per species and utilizes data that is commonly recorded during data collection for faunal assemblages, it can be variably applied for comparisons between species, sites, time periods, and even between the morphologically-identifiable and -unidentifiable portions of an assemblage. To test the approach and provide examples for application, RA has here been applied to both the ZooMS- and morphologically-identified horse (*Equus* sp.) specimens from Geißenklösterle in the Ach Valley of the Swabian Jura, Germany. Initial results indicate correlations between traditional quantification methods and RA ratios within the morphological assemblage. With additional work, RA will ideally be expanded to allow for more thorough, holistic analyses of entire faunal assemblages and will help ZooMS to reveal clues “hidden in plain sight”.

High-throughput analysis and batch effect in ZooMS MALDI-TOF data

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MALDI-TOF allows ZooMS studies to analyze hundreds or thousands of samples, either bone or parchment. The primary aim of the analysis is taxonomic identification by assessing the presence or absence of certain peak markers. These correspond to peptides that allow a certain level of taxonomic discrimination. These analyses treat the spectra and peaks in a binary or qualitative way. Differences in diagenetic processes are studied by calculating glutamine deamidation. This requires the quantitative analysis of isotopic envelopes in the spectra. Some studies are starting to look at the spectra as a whole and use spectra preprocessing techniques followed by statistical methods to cluster samples and discover markers. However, the high amount of samples in a dataset poses 2 challenges: 1) the need for streamlined pipelines that allow the high-throughput analysis of samples and 2) the impossibility of analysing all the samples in the same batch or MALDI plate. In this study, I conceptualize the analysis of ZooMS data into data-guided and marker-guided analysis. I present an R package to provide a framework to perform these types of analyses. It allows loading the data in chunks and processing them in parallel using multiple cores. This speeds up the analysis while allowing the loading and processing of thousands of samples that would not fit in the memory of a conventional computer. Moreover, I assess the batch effect in different datasets, and how it affects qualitatively, by looking at the presence or absence of peaks and quantitatively, by studying how spectra cluster together.

SpecieScan: Automated Species Identification of Bone Fragments

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Species identification in Zooarchaeology by Mass Spectrometry (ZooMS) typically involves manual analysis of matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) spectra. However, this process can be challenging, requiring training, expert judgement, and significant time and effort. Additionally, the resulting identifications may be influenced by the sequence of peptide markers examined, may not be geographically relevant, and may not provide quantitative results with confidence intervals. While there have been a few studies that have developed automated algorithms for species identification, these are generally specific to parchments or are not openly available to researchers. As the ZooMS reference database expands to new regions and with the addition of new markers and reference taxa, the need for a quicker and reproducible identification tool is needed.

In this talk we present current efforts to create an open-access algorithm for automating species identification from raw MALDI-ToF data. The algorithm was developed using Python and R programming languages. The study used MALDI-ToF data obtained at our ZooMS labs in Austria between 2022-2023, using Paleolithic bone fragments from Denisova cave and other European and Asian sites, and the algorithm was also tested using other publicly available MALDI-ToF data produced by other labs.

Initially, the raw data was preprocessed using R, for smoothing, noise removal and baseline correction, intensity calibration, peak picking, and deisotoping. The resulting triplicates were then averaged, exported, and read into Python, and the new spectrum was matched to geographically-relevant reference databases using correlation and accounting for possible deamidation. The final output of the algorithm are .csv files including the peptide markers, the closest matching species (family, order) and the similarity between unknown samples and reference data.

Results produced by the algorithm showed that they are >90% accurate on a genus-level and >95% accurate on a family-level for well preserved, internally prepared samples. It also showed >95% accuracy on a family-level for literature spectra obtained from poorly preserved bones.

