

# Special Issue: Integrating ZooMS and Zooarchaeology: Methodological Challenges and Interpretive Potentials

## Integrating Morphological and ZooMS-Based Approaches to Zooarchaeology at Vogelherd Cave in Southwestern Germany

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### ABSTRACT

Zooarchaeology is an established subfield of archaeology that incorporates a variety of interdisciplinary tools. Advances in analytical methods like radiocarbon dating, stable isotope analysis, and ancient DNA have added new dimensions to zooarchaeological research in the past century. In recent years, the addition of ZooMS (Zooarchaeology by Mass Spectrometry) has offered exciting new possibilities for studying faunal remains in archaeological contexts. In this study, we use the Vogelherd Cave, a Paleolithic site in the Swabian Jura of southwestern Germany, to showcase the advances in zooarchaeological analysis and changes in research focus. In 1931, G. Riek from the University of Tübingen completely excavated the site's rich deposits. In 2005–2012 and 2022–2023, N. J. Conard and a team from the University of Tübingen excavated Riek's backdirt using modern excavation techniques. The first systematic analysis of the faunal assemblage from a paleontological perspective was published by U. Lehmann in 1954, but it was not until the early 2000s that L. Niven undertook a comprehensive zooarchaeological study. In 2014, U. Boger and colleagues analyzed the faunal remains from the backdirt to gain a more complete view of the faunal assemblage. The current study adds the first ZooMS analysis at the site, focusing on 287 fragmentary bones obtained after water-screening the backdirt sediment. Here, we compile and compare our new ZooMS results to previous faunal datasets from Vogelherd. The history of research at the site provides a representative example of how the research focus has expanded over time and how novel analytical methods may contribute to the interpretation of an assemblage. Our ZooMS results represent the taxonomic abundance in a moderate way, which falls between the morphologically identified results of Niven and Boger et al. By juxtaposing traditional zooarchaeological results and ZooMS data, we explore the strengths and weaknesses of each approach and contemplate how best to integrate these methods in future research.

## INTRODUCTION

Zooarchaeology is a multidisciplinary field that involves the study of animal remains from archaeological sites to reconstruct human-animal interactions in specific environments (Reitz and Wing 1999). This type of research initially originated alongside prehistoric archaeology. In the mid-19th century, faunal studies began to focus on domestic animals or modified bone tools, leading to cultural interpretations (Eaton 1898; Forchhammer 1852; Mills 1904). Typically, zoologists or paleontologists conducted these early faunal studies. In the 1950s, guidelines on animal bone sorting and identification for archaeologists became available (Cornwall 1956; Lawrence 1951). Zooarchaeological research evolved beyond species lists to a dynamic field investigating all aspects of past human-animal-environment interactions. In 1971, Olsen (1971) proposed the term “zooarchaeology” to describe the study of animal remains to answer archaeological questions. Following this, zooarchaeological quantitative methods and report paradigms were developed and published (Brumley 1973; Grayson 1979; Grigson 2016). Discussions about the identification of cutmarks and fragmentation patterns on bones, such as those conducted by Behrensmeier and colleagues (1986), Binford (1981) and Johnson (1985), have contributed to the methodology of zooarchaeology. Archaeology has also updated the fieldwork standards and more widely adopted practices, such as sediment screening (Geiling et al. 2018), that provided enlarged faunal assemblages for zooarchaeological studies.

The application of molecular analytical methods, such as isotopes, radiocarbon dating, and ancient DNA (aDNA), has enriched the zooarchaeological endeavor by providing direct information about age, diet, and phylogenetics. aDNA analysis is an effective tool for assigning morphologically tricky specimens and understanding the history of animal domestication (Horsburgh 2008; Librado et al. 2021). In 2009, a paleoproteomics method, ZooMS (Zooarchaeology by Mass Spectrometry), was introduced as a taxonomic identification tool (Buckley et al. 2009). Due to its low cost and potential for high throughput, ZooMS offers a sustainable solution to taxonomically identifying large numbers of fragmented bones from archaeological sites. ZooMS outperforms other taxonomic techniques focusing on fragmented bones, e.g., macroscopical observation (Cuijpers 2006) or metabarcoding DNA (Greally et al. 2015), and provides extensive taxonomic data for zooarchaeology (Brown et al. 2021; Buckley et al. 2017; Martisius et al. 2022; Pothier-Bouchard et al. 2020; Ruebens et al. 2023; Sinet-Mathiot et al. 2023, 2019; Torres-Iglesias et al. 2024). However, concerns about the way of integrating traditional zooarchaeological results and ZooMS datasets are being raised (Banning 2020; Giovas and LeFebvre 2017).

In this study, we examine the evolutionary trajectory of the field of zooarchaeology through the lens of the Vogelherd Cave case. The archaeological studies at Vogelherd have produced a wealth of information regarding the prehistoric occupants and their choices, and the paleoecological framework they existed in. Starting with a brief exca-

vation history of Vogelherd (Figure 1), we introduce the cultural attributions of the original deposit in the cave. Subsequently, we summarize the three traditional zooarchaeological studies of Vogelherd’s faunal collections, in terms of methods, results, and representative conclusions they each reached. We then present our latest results based on the application of ZooMS on a small faunal sub-set of Vogelherd. Finally, we undertake a comparative analysis of the four zooarchaeological studies, delving into their potential for integration. The paper provides a historical overview of the development of zooarchaeology, since each dataset represents a typical research of its time.

## VOGELHERD CAVE EXCAVATION HISTORY

Vogelherd Cave is part of the karst system in the Swabian Jura of southwestern Germany. Located in the Lone Valley, between the Danube River to the south and the Jurassic Plateau to the north, the cave, with its three entrances, covers an area of ca. 170m<sup>2</sup>. This site provides outstanding views of the Lone Valley and has a comfortable scale, making it a desirable location for human habitation.

Gustav Riek from the University of Tübingen and a small team of local workers excavated the site in twelve weeks during the summer of 1931 and removed around 500m<sup>3</sup> of deposits. Riek documented twelve stratigraphic profiles and identified nine cultural horizons spanning the Neolithic to the Middle Paleolithic, which he published in his famous monograph in 1934.

The Archaeological Horizon I (AH), specifically assigned to the Neolithic period. AH II and III, deposited in the early phase post the Last Glacial Maximum, are characterized as the Magdalenian. The Magdalenian horizons at Vogelherd, as inferred from low find density, suggested short-time stays at Vogelherd (Niven 2006; Riek 1934).

Riek described no Gravettian layer at Vogelherd. Nevertheless, subsequent archaeological investigations identified potential Gravettian blades and points, and the radiocarbon date on a bone from AH IV fell within the Gravettian period (ca. 26 ka), suggesting that there was Gravettian component at Vogelherd (Conard and Bolus 2003; Conard et al. 2012). AH IV and V were referred to as the “upper and middle Aurignacian” by Riek (Riek 1934). Radiocarbon dates from the two Aurignacian horizons at Vogelherd, and corroborating evidence from other Swabian Jura sites firmly establish them as among the earliest Aurignacian technocomplexes in Europe, dating to ca. 43–35 ka (Conard and Bolus 2003; Conard et al. 2004; Higham et al. 2012).

Originally designated as the “lower Aurignacian,” AH VI was later re-assigned to the Middle Paleolithic (Müller-Beck 1957). AH VII and AH VIII were similarly attributed to the Middle Paleolithic by Riek. Excavating down to the bedrock, Riek assigned AH IX as the “culture of the cave floor” (Riek 1934). This horizon, composed of ochre-yellow loam and bean ore, likely dates back to Marine Isotope Stage 5e, a period of warmer climate, a conclusion supported by the recovery of a molar tooth from a forest elephant that thrived in such conditions (Niven 2006).

The Aurignacian horizons AH V and IV represent by

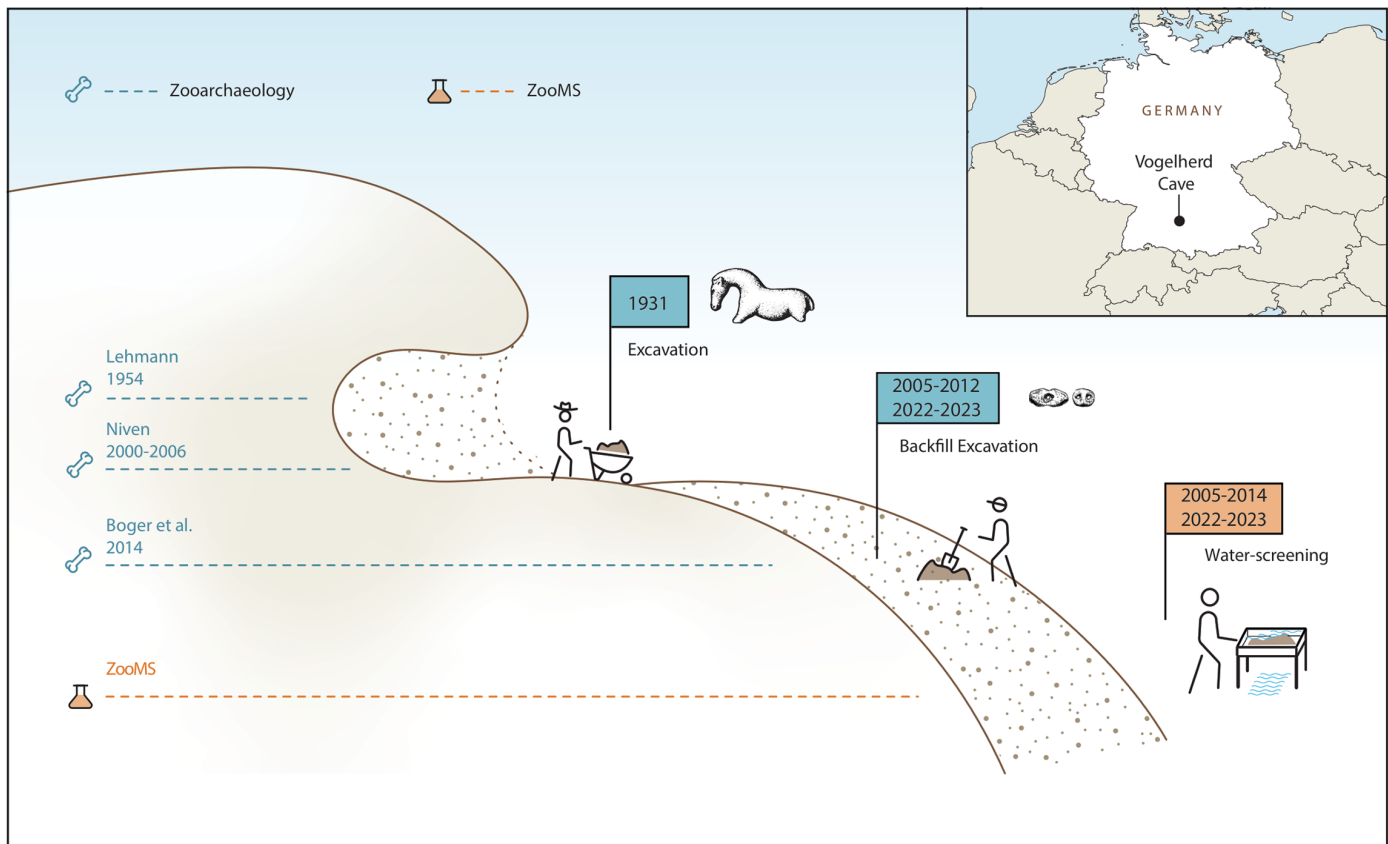


Figure 1. The schematic of Vogelherd Cave highlights two main excavations and the four faunal assemblage studies.

far the richest layers at Vogelherd and yielded 2,863 lithic tools and thousands of blanks, as well as nine ivory figurines and numerous other organic artifacts (Hahn 1977; Riek 1934). While the excavation from 1931 lacked piece-plotting, careful scrutiny of the documentation and labeled finds sometimes provides a degree of contextual information beyond the stratigraphic attribution, the refits of lithic and faunal material show a degree of mixing between the layers (Schürch in prep.).

In addition to the lithic and other artifact assemblages, Riek's excavation produced more than 18,000 bones, making this one of the largest Upper Paleolithic faunal assemblages in Central Europe (Niven 2007). Along with other human remains, Riek recovered a modern human skull, known as the Stetten 1 cranium, from the base of Aurignacian Horizon V. This fossil long served as evidence for assigning the site's figurative art to modern humans (Riek 1934). Direct AMS dating of the Stetten 1 cranium and other human skeletal material, however, revealed that they were of Neolithic age, approximately 5,000 years ago (Conard 2009; Conard et al. 2004).

In order to gain additional information and contextualize the results from Riek's dig, from 2005 to 2012 and 2022 to 2023, a team from the University of Tübingen under Nicholas J. Conard's direction re-excavated the backdirt from the original fieldwork on a slope outside Vogelherd. Conard's team excavated the entire volume of sediment from the cave bucket by bucket while piece-plotting nu-

merous single finds and then water-screening all the sediments. As a result, the team recovered over 200,000 lithic artifacts, hundreds of Aurignacian ivory beads, numerous fragments of figurative artworks and musical instruments, and countless unidentified fragments of osseous material (Conard et al. 2015a).

## ZOOARCHAEOLOGICAL STUDIES AT VOGELHERD CAVE

### THE FIRST VOGELHERD FAUNAL ANALYSIS OF THE 1931 EXCAVATION

*"The Vogelherd offers the researchers the great advantage as it was carefully excavated horizontally and well-documented."*  
(Lehmann 1954: 144)

Paleontologist Ulrich Lehmann conducted the first study of the 1931 Vogelherd faunal assemblage. In his publication, Lehmann appreciated the methods and recording of Riek's excavation.

Although some contextual information was available, the results were listed taxonomically in Lehmann's publication. Lehmann only examined intact fossils. He identified species and body sizes, based on teeth and the more complete bones that were identifiable and measurable. The intact fossils, however, constituted only a fraction of the

assemblage collected at the site (n=921). Morphology and morphometrics dominated Lehmann's study, reflecting his paleontological interest. He compared the morphometric data within Vogelherd using available data from other sites and modern collections (illustrated in tables and line charts). The statistical differences showed changing trends, and Lehmann suggested that the size of animals reflected an adaptation to paleoecological changes. Lehmann identified all 921 specimens to at least genus level, with even subspecies identification for equids (Table 1); he did not mention fragmentary and unidentifiable specimens. The paleontologist also excluded the Elephantidae remains, which comprised the most significant proportion of the Vogelherd faunal assemblage. These remains were dispatched to another Elephantidae paleontologist, Karl Dietrich Adam, whose work was never published. Lehmann only cited Elephantidae's minimum number of individuals (MNI) based on Adam's study. MNI has a more notable position than the number of identified specimens (NISP) in Lehmann's study, where he listed the MNI values of each taxon in a table but only included NISP values in the description text. Element counts were not fully reported, and how the MNI values were derived was also unclear.

At the end of the publication, Lehmann (1954) attempted to reconstruct the paleoclimate by comparing taxonomic abundances and body size differences among cultural horizons. He concluded that the Middle Paleolithic period at Vogelherd was cool and the Upper Paleolithic (Aurignacian and Magdalenian) was colder, due to the presence of more Arctic fauna; however, the climate at Vogelherd was warm prior to the occupation of hominin. This climate reconstruction on fauna was generally correct through later climate reconstruction research on other proxies (Andersen et al. 2006; Rasmussen et al. 2014). The last glacial maximum between Aurignacian and Magdalenian occupation left almost no fauna fossils at Vogelherd, making it hardly detectable for Lehmann. Regarding the formation of the Vogelherd deposit, Lehmann assumed that hominins had introduced the faunal remains to the cave, so it is not a carnivore den or natural trap. He concluded that horses, mammoths, and reindeer were the main prey targets of the occupants at Vogelherd, based on their high MNI values.

Unlike a species list (Lyman 2015a) that was common in his time, Lehmann's study is closer to what was later defined as zooarchaeology. He published his work on its own, not as an appendix in a monograph; he quantified and produced exact values of MNI and NISP, rather than describing them as "rare" or "common." Although modifications were beyond the scope of his paleontological research, he also observed and recorded a few modification traces as cutmarks on reindeer and wolf remains. Finally, he wrote two sections on interpreting human subsistence strategies, and the study ends with the relationship between ancient humans and their ecological context.

## THE COMPLETE ZOOARCHAEOLOGICAL STUDY OF THE 1931 FAUNAL ASSEMBLAGE

*"Initially, an attempt was made in this study to match specific finds described or depicted in Lehmann's report with those in the existing collections." (Niven 2006: 7).*

Between 1999 and 2004, zooarchaeologist Laura Niven studied the fauna from the 1931 excavation as a doctoral candidate in the German Science Foundation's Collaborative Research Centre 275: Climate-coupled processes in the Mesozoic and Cenozoic eras based at the University of Tübingen. Her work, which was supervised by Nicholas J. Conard and Hans-P. Uerpmann, represents the first comprehensive study of the faunal material from the Vogelherd Cave (Niven 2001, 2003, 2006, 2007). During her initial attempts to match the specimens with Lehmann's descriptions, Niven found it challenging to locate catalog numbers after nearly 50 years. At the time of Niven's research, the Vogelherd faunal collection was housed at four locations. The cataloged faunal remains comprised around 18,800 specimens, including osseous tools and artifacts. Among these, 14,181 specimens were >1cm long and preserved stratigraphic information. These 14,181 findings formed the central focus of Niven's study. According to Niven (2006), ca. 94% of the studied specimens (n=13,282) were from the Aurignacian horizons. However, the overlap with Lehmann's study was not exact, given loss, curatorial breakage, and refits over 50 years. Lehmann's earlier study occasionally documented specimens in more complete preservation than later studies.

Niven's study assessed the completeness of the 1931 excavation by quantifying the types of long bone circumferences and determining the minimum number of elements (MNE) in the Aurignacian assemblage (Niven 2006). The results showed that, unlike other early excavations that often discarded long bone shafts (Marean 1998; Marean et al. 2004), bones in Vogelherd Aurignacian horizons were equally collected, except those <3cm in length. The collection completeness analysis laid a strong foundation for Niven's subsequent reconstruction of human behavior at the site.

Niven (2006) taxonomically determined 7,730 specimens to family, genus, or species (see Table 1). For the unidentified fragments, Niven classified them using broader designations such as "large artiodactyl" or by body sizes. In addition to Lehmann's taxa list, Niven described a few bird species (n=13) for the first time in the Aurignacian context. Mammoths accounted for 46% of Niven's identifiable assemblage. Niven suggested that Aurignacian humans collected large quantities of mammoth bones and ivory not for food, but for other particular uses. Thus, mammoths were not regarded as one of the main prey at Vogelherd (Niven 2001, 2006).



**TABLE 1. TAXON TALLIES AND COMPOSITIONS OF ZooMS, ARCHAEOLOGICAL LAYERS AH II-IX BY LEHMANN (1954), NIVEN (2006), AND GEOLOGICAL UNIT HL/KS (Boger et al. 2014) (continued). (Note that the bold cells refer to ZooMS-only categories.)**

Categories	Identification		ZooMS		Morphological approach					
			counts	%	AH II-IX (Lehmann 1954)		AH II-IX (Niven 2006)		HL/KS (Boger et al. 2014)	
	Taxon				NISP	%	NISP	%	NISP	%
Elephantidae	<i>Elephas antiquus</i>				1	0.11	1	0.01		
	<i>Mammuthus primigenius</i>						3585	46.38	686	34.88
	<b><i>Mammuthus primigenius/Elephas antiquus</i></b>		79	32.24						
		Sub-total	79	32.24	1	0.11	3586	46.39	686	34.88
Canidae	<i>Canis lupus</i>				29	3.15	45	0.58	74	3.76
	<b><i>Canis lupus/Vulpes lagopus</i></b>		2	0.82						
	<i>Vulpes vulpes</i>		6	2.45	2	0.22				
	<i>Vulpes lagopus</i>				5	0.54				
	<i>Vulpes sp.</i>						20	0.26	102	5.19
			Sub-total	8	3.27	36	3.91	65	0.84	176
other carnivores	<b>Felidae/Hyaenidae/Mustelidae</b>		2	0.82						
	<b>Mustelidae</b>		4	1.63						
	<i>Meles meles</i>				3	0.33		excluded	188	9.56
	<i>Gulo gulo</i>				2	0.22	1	0.01	1	0.05
	<i>Martes sp.</i>								5	0.25
	<i>Mustela sp.</i>								1	0.05
	<i>Panthera leo spelaea</i>				4	0.43	6	0.08	2	0.10
	<i>Crocuta crocuta spelaea</i>				12	1.30	27	0.35	27	1.37
	<i>Felis silvestris</i>						3	0.04	36	1.83
			Sub-total	6	2.45	21	2.28	37	0.48	260
	<b>unknown for ZooMS</b>		2							
	failed identification		42	14.63		not available	5620	42.10	375	16.01
	Total	287		921			13350		2342	

Unlike Lehmann, Niven is a zooarchaeologist. This background difference is reflected in the organization of their publications. In the initial part of Niven's (2006) book, the author explained the zooarchaeological methods she would employ, including the definitions for quantification units and anthropogenic and non-anthropogenic modifications. She also briefly introduced the history and paleoecology of the mammal species found at Vogelherd. The author presented the study in a way most relevant to addressing archaeological interests, in chronological sequence—Middle Paleolithic, Aurignacian, and Magdalenian. She evaluated the properties of each cultural deposit, whether carnivores or hominins were the main accumulators, with multiple zooarchaeological proxies including standard quantification units (e.g., NISP, MIN, MNE, and MAU), modification, age, and sex profiles of the studied fauna. Niven also measured the morphometric data for establishing taxa age profile and season-at-death. In the last chapter, she places the Vogelherd faunal assemblage in the context of the Lone Valley and the Swabian Jura and summarizes the human subsistence behavior (Niven 2006).

### THE PLOTTED FAUNAL ASSEMBLAGE FROM VOGELHERD BACKDIRT

*"The excavated sediments were all screened, and further insights into small game exploitation will be revealed after analyzing these remains"* (Boger et al. 2014).

From 2005 to 2012 and 2022 to 2023, a team from the University of Tübingen under Nicholas J. Conard's direction re-excavated the backdirt from the original fieldwork on a slope outside Vogelherd Cave. The 2005–2012 dig cleared nearly 90% of the sediment previously in the cave. Conard's team excavated sediment by bucket unit, while piece-plotting numerous single finds that were visible in the field. Following the first re-excavation of the backdirt, Boger and colleagues (2014) analyzed these plotted faunal remains.

Given that reconstructing the history of a backdirt formation is not always feasible (e.g., Wright et al. 2021) and rarely reliable, Boger et al. (2014) examined the backdirt faunal assemblage (n=2,342) as a whole, regardless of the bone locations in the backdirt. As Niven (2006) claimed that 94% of the faunal remains in the 1931 excavation came from intact Aurignacian horizons, Boger and colleagues assumed a similar proportion of Aurignacian remains in the 2005–2012 handpicked assemblage. Moreover, they tried to assign the faunal remains to their original context (Middle Paleolithic, Aurignacian, or Magdalenian) based on the state of preservation. However, this was not possible because of the variability of bone preservation and differences between *in situ* finds and the backdirt assemblage (Boger et al. 2014).

Boger et al. (2014) identified nearly 84% of the handpicked assemblage to genus level or higher (see Table 1), representing a high identification rate for *in situ* Paleolithic horizons in the Lone Valley (Kitagawa 2014). For the unidentified specimens (n=375), Boger et al. (2014) assigned

them to four body size groups following Brain's (1983) classification. Regarding faunal diversity, this study added a few new taxa to Vogelherd's previously known faunal list. These new taxa included small mammals such as roe deer, marten, polecat, and hedgehog (n=93). Intrusive animals, such as badgers (n=188), and domesticated animals like sheep/goats (n=25), were excluded in Niven's study, but included by Boger et al. (2014). These species, however, likely played little or no role in the subsistence of the Paleolithic inhabitants of Vogelherd Cave.

Regarding species abundance through NISP values, the most noticeable difference compared with the *in situ* assemblages was the higher percentage of small animals, such as wild hares, in the backdirt. Clearly, Riek's excavation overlooked much of the small and highly fragmented faunal material, which resulted in a less prominent presence of the two main prey species, horse and reindeer, in the backdirt assemblage. The composition of mammoths remained similar, but in the backdirt, mammoths were notably over-represented by ivory fragments, which accounted for 94% of the total NISP for mammoths. Retrieved from the backdirt, the generally smaller size of specimens attributed most of the differences observed in the comparison.

Results in Boger et al. (2014) reflected a heightened interest in the potential role of the small and low-ranked game, especially wild hares (n=106), with emphasis on examining diet breadth and resource ranking during the Paleolithic. They also argued that water-screened sediments would reveal evidence of a higher level of small animals (Boger et al. 2014). This prediction becomes testable when we apply ZooMS to the highly fragmented faunal remains recovered during water screening.

### ZOOMS-ANALYZED WATER-SCREENED BONE FRAGMENTS FROM VOGELHERD

ZooMS is a paleoproteomics approach providing a low-cost, fast, and reliable way of speciating collagen materials such as bone, ivory, and leather. Collagen is the major organic component (~90%) in the bone of vertebrates, and its survival was tested in many Paleolithic sites (Richter et al. 2022). ZooMS involves extracting Type I collagen (COL1) from a sample and generating tryptic-digested peptide mass fingerprints via MALDI-TOF-MS (Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry).

Sediments excavated in 2005–2012, collected from over 32,000 buckets, were water-screened using a 2mm sieve. This work was completed by 2014, two years after the excavations concluded (Conard et al. 2015a). By sorting the dry sediment, identifiable microfauna bones, teeth/ivory fragments, burnt bones, and tiny artifacts like ivory beads were separated from highly fragmented bones. The sorting resulted in >100kg of bone fragments, stored in bags and recorded with bucket units (Conard et al. 2015b; Schuerch et al. 2021). Conard's team found hundreds of ivory beads in the backdirt, echoing the discovery of an ivory rod cache in the 1931 excavation (Riek 1934), likely for bead-making. Most beads have similar characteristics to those from other

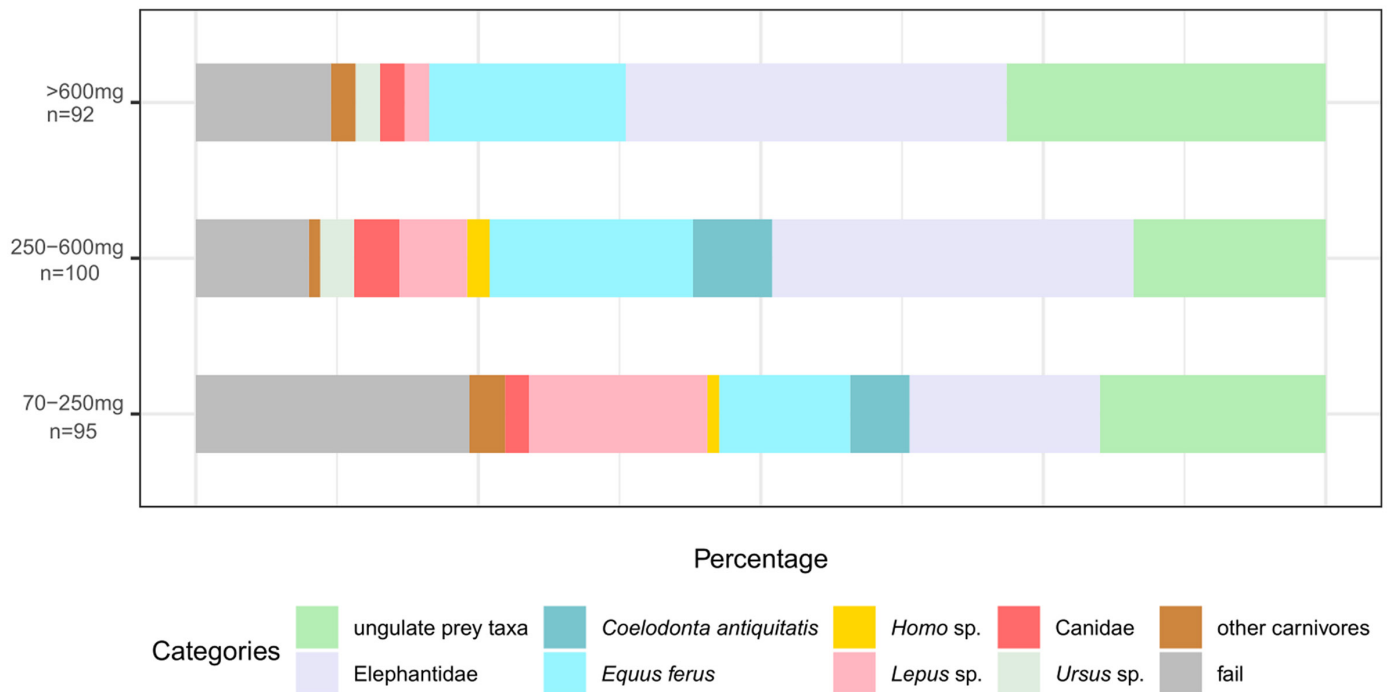


Figure 2. ZooMS identifications grouped in weights. Group 70–250mg, n=95; Group 250–600mg, n=100; Group >600mg, n=92.

Swabian Jura caves dating to the Aurignacian period (Hahn 1988; Wolf and Conard 2015).

To apply ZooMS in this assemblage, we selected random bags of bones from buckets containing ivory beads. The sampled material came from the front of the southwestern entrance of Vogelherd Cave (Supplementary Material Figure S1). We sampled bones with initial weight >70mg for ZooMS (n=287), since smaller bones would preclude subsequent analyses, such as radiocarbon dating, aDNA, or stable isotopes. Most of the ZooMS samples measured between 1–2cm in length. A bone chip of ~20mg was removed from each bone fragment for ZooMS analysis using published ZooMS protocols (Brown et al. 2020; Buckley et al. 2009) (details on the applied ZooMS protocol can be found in the supplementary text). We identified the spectra using published ZooMS reference data (Buckley and Kansa 2011; Buckley et al. 2009; Janzen et al. 2021; Welker et al. 2016).

Despite being buried outside the cave for nearly 70 years, 85% of the 287 specimens preserved collagen for ZooMS identifications. Of the 287 samples, 202 were identified at least to the genus level, while 41 had more generic assignments (see Table 1). The ZooMS assemblage is much smaller than the plotted finds in previous studies; hence, no new species were identified. The ZooMS water-screened assemblage revealed a significantly higher number of hares, echoing the observations made by Boger et al. (2014). In addition, we discovered three new hominin fossils. All three fragments were small, weighing between 170mg and 407mg. Direct radiocarbon dating and aDNA analysis suggest they belonged to at least two individuals who lived in different periods, Magdalenian and Neolithic (Wang et al. authors' unpublished results).

We expected that the ZooMS data would illuminate the fragmentation patterns to some degree. To test this, we divided the 287 specimens into three weight groups: 95 specimens weighing between 70–250mg, 100 specimens weighing 250–600mg, and 92 specimens weighing >600mg (Figure 2). The lightest group includes most specimens that failed collagen extraction, indicating relatively poor COL1 preservation. Hares (*Lepus* sp.) mostly weigh less than 600mg, and bear (*Ursus*) are absent in the 70–250mg range, reflecting their respective body sizes. In contrast to our expectations, woolly rhinoceros (*Coelodonta antiquitatis*) are absent in the heaviest group. No qualitative correlation was observed between the counts and the specimen weights for dominant taxa such as horse, ungulate, and Elephantidae.

#### COMPARISON OF THE FAUNA DATASETS

The four zooarchaeological studies of Vogelherd presented here are distinct in their own ways. Lehmann's (1954) study focused only on the complete and identifiable portion of the 1931 fauna. Niven (2006) later re-examined the entire 1931 faunal collection using standard zooarchaeological approaches. Following the excavation of Vogelherd backdirt, Boger and colleagues (2014) analyzed the piece plotted fossils from the backdirt and compared them with the *in situ* assemblage. Here, we tested 287 bone fragments from the water-screened backdirt using ZooMS.

NISP and MNI are the fundamental quantitative units in zooarchaeology. NISP represents the most straightforward observational measure of taxonomic abundance, while MNI values derive from quantitative units depending on element identifications. There has been a long-standing debate regarding the advantages and disadvantages of



TABLE 2. COMPARISON OF HORSE (*Equus*) AND REINDER (*Rangifer tarandus*).\*

	NISP				MNI		
	AH II-IX (Lehmann 1954)	AH II-IX (Niven 2006)	HL/KS (Boger et al. 2014)	HL/KS ZooMS (this study)	AH II-IX (Lehmann 1954)	AH II-IX (Niven 2006)	HL/KS (Boger et al. 2014)
<i>Equus sp.</i>	586	1825	229	45	52	61	13
<i>Rangifer tarandus</i>	115	1679	123	28	18	35	6
<i>Equus / Rangifer sp. ratio</i>	5.10	1.09	1.86	1.61	2.89	1.74	2.17

\*AH means *in situ* archaeological horizon and HL/KS refers to the backdirt. Data from Boger et al. 2014; Lehmann 1954; Niven 2006.

MNI over NISP in zooarchaeological studies (Brothwell and Chaplin 1972; Domínguez-Rodrigo 2012; Grayson 1979; Lyman 2018; Marshall and Pilgram 1993; Morin et al. 2017; Uerpmann 1973). Most zooarchaeological studies list both NISP and MNI values in their taxonomic table (Niven 2006, 2007). However, there has been an emphasis shift from MNI to NISP values in the past decades (Lyman 2018). This trend is evident in the research of Vogelherd; Lehmann (1954) only listed MNI values in the taxonomic table, while Boger et al. (2014) listed NISP values only. This trend is compatible, and somehow beneficial to ZooMS studies since ZooMS identifications can only result in NISP counts.

Previous studies showed that although mammoth remains make up a large composition in the Vogelherd fauna, horses and reindeer are two primary prey taxa at Vogelherd. According to Niven (2007), Aurignacian people hunted both taxa seasonally, likely transporting complete carcasses back to the cave. The NISP and MNI values of horses and reindeer established in previous studies, and the ZooMS counts from this work, are used in a comparison (Table 2). As demonstrated previously (Lyman 2019), NISP values consistently correlate with MNI values across various studies—horses outnumber reindeer in both NISP and MNI (horse/reindeer ratios >1). Notably, while the data from Lehmann and Niven represent independent analyses of the same collection obtained from the 1931 excavation, their ratios display the largest difference (5.10 vs. 1.09). Conversely, assemblages derived from backdirt plotted and water-screened exhibit closer horse/reindeer ratios (1.86 vs. 1.61) (Boger et al. 2014; Lehmann 1954; Niven 2006).

In traditional zooarchaeological studies, body size classification, based on bone cortical thickness and fragment size (Brain 1983), is frequently used to group morphologically unidentifiable bone fragments. In the case of Vogelherd, both Niven (2006) and Boger et al. (2014) assigned unidentifiable specimens into body size groups and provided the tallies in their publications. Body size 5 includes mammoth and rhinoceros; body size 4, horse/bear/

red deer; body size 3 includes reindeer/roe deer; and, body size 2 includes fox/hare (Boger et al. 2014; Niven, 2006). In Figure 3, we also convert the ZooMS result to body size groups for comparison. Based on more specific ZooMS assignments, the body size classification of the ZooMS assemblage is accurate and unbiased. We find that the body-size-based groups of Niven (2006) and Boger et al. (2014) (the outer and middle rings) show similar abundance patterns, especially for body sizes 3 and 4, suggesting that middle-sized animals dominated the non-diagnostic specimens. However, the ZooMS assemblage, which stems from highly fragmented specimens, shows a different pattern. Large-sized animals (body size 5) dominate the ZooMS assemblage and small-sized animals (body size 2) are also more abundant when compared to the other two datasets. The two body size patterns may be the result of actual differences between plotted and water-screened assemblages, or, as shown in other ZooMS-based studies, body size classes based on cortical thickness do not accurately reflect the overall species composition at a site (Sinet-Mathiot et al. 2019; 2023; Torres-Iglesias et al. 2024).

To better understand these differences, we convert the ZooMS dataset in taxonomic categories and compare it with the identifiable sub-assemblages from the previous three studies (Boger et al. 2014; Lehmann 1954; Niven 2006) (Figure 4). The broad categories, rather than precise taxa, are used in the comparison. Humans, hedgehogs (*Erinaceus europaeus*), and birds are absent in more than one dataset, and thus are excluded in this new comparison. Furthermore, NISP values from the three richest contexts, AH III, AH IV-V and AH VII, corresponding to the Magadalenian, Aurignacian and Middle Paleolithic, respectively, are extracted from Niven's dataset and shown in the bar chart of Figure 4a.

The assemblages of Niven (2006), Boger et al. (2014), and ZooMS have more complete categories, thus, we conducted a Chi-square test of independence between these three. The results show a significant difference in their

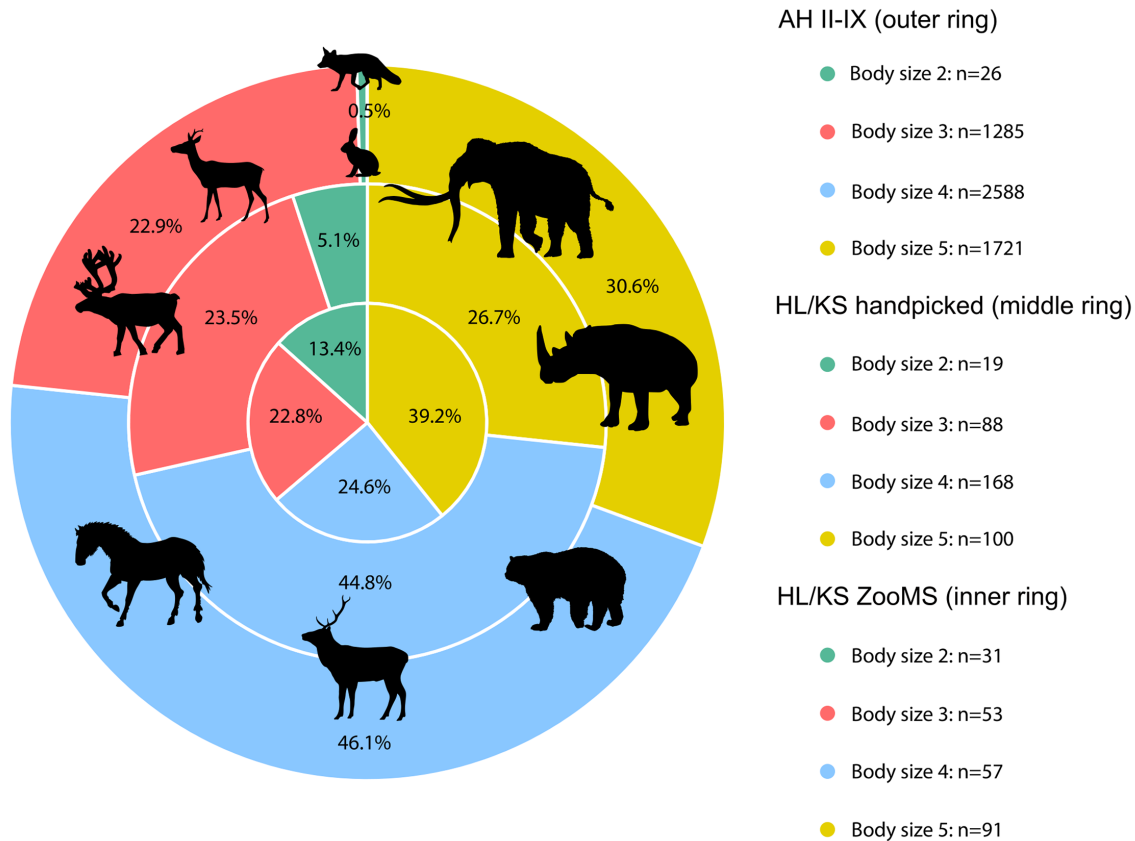


Figure 3. Body-size based groups and compositions of the morphologically unidentifiable bone remains at Vogelherd. The outer ring, AH II-IX, represents *in situ* data from Niven (2006); the middle ring shows data from the backdirt handpicked bones (Boger et al. 2014); the inner ring shows data from the ZooMS analysis of backdirt water-screened bones.

taxonomic profiles ( $\chi^2 = 1924.6$ ,  $df=14$ ,  $p < 0.01$ ), suggesting that the proportion of each category is not constant across assemblages, as shown in Figure 4a. Referring to the horse/reindeer ratio in these three assemblages, we would have expected the ZooMS taxonomic abundance to be closer to that of Boger et al.'s (2014) assemblage, as they are both based on materials from the backdirt. However, this is not the case. The chi-square standardized residuals (Figure 4b) of categories in Boger et al. (2014) are the highest and contribute the most to the difference. The ZooMS assemblage has the smallest residual contribution, which means it falls between the assemblages of Niven (2006) and Boger et al. (2014) and is more towards Niven's (2006).

Ivory and teeth fragments, both contributing significantly to the NISP values of mammoth and horse (67% and 52% in Niven's [2006] assemblage), are not part of the ZooMS dataset since only bones were studied. Hence, the similarity of the ZooMS-based assemblage with Niven's could be indeed higher. A distinct difference between the ZooMS and Niven's datasets is the identification of more hares and carnivores (except for bears) (see Figure 4b) in the ZooMS assemblage. We know that due to the speed of the operations and the focus on larger mammals and human remains, there were clear collecting biases against small

animals in the 1931 excavation. Figure 2 has confirmed that hare bones are highly fragmented, mostly found in the 70–250mg weight group. Boger et al. (2014) also noted the large number of small game remains from the backdirt plotted assemblage, and they recorded clear human modifications on hare remains. Given the high fragmentation of hare remains in the water-screened assemblage, we suggest that small game, such as hares, may have played a more significant role in the subsistence strategies of the Aurignacian inhabitants of the site.

We should not expect an identical taxonomic abundance in ZooMS and morphologically identified assemblages. While coming from the same deposit, the ZooMS bones were small in size and morphologically undiagnostic. On the one hand, we can assume that counts achieved by ZooMS positively correlate with the body sizes of taxa (Brown et al. 2021). On the other hand, we may also suggest that intensive fragmentation is less likely to occur on larger mammal skeletons (Cannon 2013). Nevertheless, the abundance of a species in a zooarchaeological collection can never be assessed reliably in absolute terms (Lyman 2018).

Finally, we find Lehmann and Niven's abundance patterns unrelated, even after removing the mammoths from Niven's assemblage (Supplementary Material Figure S2).

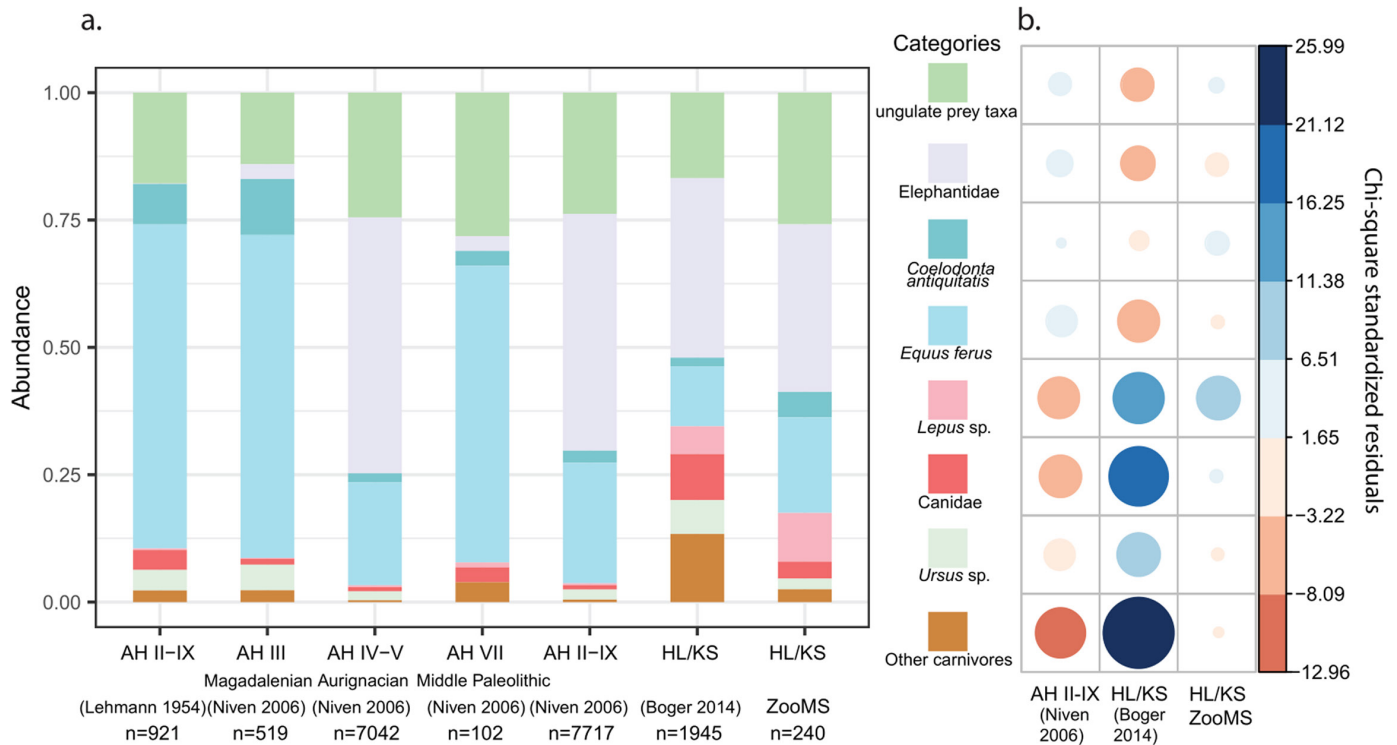


Figure 4. a) Abundance of identifiable bones by taxonomic categories, using NISP values. b) Visualization of chi-square standardized residuals of each category (data from Boger et al. 2014; Lehmann 1954; Niven 2006).

Although both assemblages are from the 1931 collection, Lehmann only examined a portion of it, while Niven examined the total of available fauna. This is a warning sign for the potential bias in partial sampling of an assemblage. Moreover, the work of Boger and colleagues stands out from the two previous analyses by a much higher identification rate (nearly 84%) of its assemblage.

## DISCUSSION

ZooMS offers new opportunities for the taxonomic identification of traditionally undiagnostic bones. In recent years, diverse approaches to integrating ZooMS and zooarchaeological datasets have appeared in the literature (Brown et al. 2021; Ruebens et al. 2022; 2023; Silvestrini et al. 2022; Sinet-Mathiot et al. 2019; 2023). These case studies are tailored to the characteristics of the individual assemblages analyzed. However, as ZooMS data accumulates, methods for quantitative integration of assemblages analyzed using traditional and biomolecular approaches are becoming more pressing. At Vogelherd, four zooarchaeological studies were conducted using both morphological and molecular (ZooMS) approaches. Hence, the site can serve as a case study for exploring ways of integrating such datasets. Any integration attempt should rely on the understanding of both approaches, their benefits and limitations. In Table 3, we list a series of points regarding zooarchaeological analyses, mostly concerning Paleolithic sites, and then try to assess them from both ZooMS and traditional zooarchaeology perspectives.

**Initial assessment.** To evaluate the feasibility of ZooMS on a certain faunal collection, a small-scale test of collagen preservation is usually a priority. If the preservation allows, the analysis can be scaled up.

Whether in ZooMS or morphological approaches, researchers are concerned with the property of the studied assemblage. Usually, the ZooMS assemblage is the unidentifiable portion eliminated from the zooarchaeological morphological identification. Thus, a combination of the two approaches should bring a nearly complete understanding of taxonomic abundance. Sometimes, the situation may be more complex due to the intentional or unintentional preselection of samples. For example, in the Vogelherd ZooMS assemblage, ivory, teeth, burnt bones, and tiny bones <70mg are excluded from being analyzed. During the subsequent integration of datasets, we should consider the properties of distinct assemblages.

**Preparation.** Before examining a collection, compiling a list of potential taxa according to regional faunal studies is necessary for a zooarchaeologist. ZooMS researchers do the same listing, but mostly on a continental/biogeographical scale. Both ZooMS and morphological approaches rely on reference databases or collections; ultimately, our identifications are just as good as our reference collections are, and we can only determine a taxon if this exists in our comparative collection or reference database. For ZooMS, reference of common species is widely accessible and has less intra-species variation (at least for mammals) (Richter et al. 2022). On the other hand, zooarchaeological reference

**TABLE 3. METHODOLOGICAL COMPARISON OF ZooMS AND TRADITIONAL ZOOARCHAEOLOGICAL APPROACHES.**

	Questions (mainly related to Paleolithic sites)	ZooMS	Zooarchaeology (morphological approach)
Initial assessment	pilot study	small-scale testing to assess collagen preservation	not necessary
	specimen selection	>70mg (to allow for subsequent analysis if an interesting species is identified). Avoid sampling bone tools or worked bones with destructive protocols	often piece plotted finds, in most cases >3cm (except microfauna)
	sampling bias	preselection of small fragments or non-diagnostic fragments	entire or partial assemblage analyzed
Preparation	reference database	published or in-lab peptide markers for species identification	reference skeletal collections and publications
	possible species list	continental fauna list (more tolerance to unexpected species)	regional fauna list and reference skeletal collections
Identification	basis of ID	collagen peptide mass fingerprinting	characteristic morphological elements
	difficult samples	clustered by collagen proximity on amino acid sequence	clustered by morphological proximity
	ID taxonomic resolution	various among different families, generally family-genus level.	various among different families, generally species level or better
	ID success rate	depending on collagen preservation	depending on fragmentation level
	analyst's bias	minimal	experience-dependent
	processing time	high-throughput, hundreds of samples per week	experience-dependent
	open data and format	published MALDI-TOF spectra	published description, photos, 3D scan, and morphometric data
	quantification	direct ZooMS counts (NISP-like)	Quantification units: NISP, MNE, MAU, MNI etc.

collections offer an unparalleled diversity of (sub-)species accumulated by generations of scholars (Driver et al. 2011).

**Identification.** ZooMS outperforms traditional zooarchaeology in identifying bone fragments lacking diagnostic features, and the generally non-targeted reference database gives ZooMS an advantage in finding unexpected taxa.

Regarding the accuracy of identification, the resolution of ZooMS is generally lower than that of morphological studies. However, with well-preserved COL1, ZooMS is able to differentiate morphologically similar species, e.g., sheep/goat, bison/buffalo, and donkey/horse (Buckley et al. 2010; Coutu et al. 2021; Jeanjean et al. 2023; Paladugu et al. 2023).

**TABLE 3. METHODOLOGICAL COMPARISON OF ZooMS AND TRADITIONAL ZOOARCHAEOLOGICAL APPROACHES (continued).**

	Questions (mainly related to Paleolithic sites)	ZooMS	Zooarchaeology (morphological approach)
Data interpretation	fragmentation level	correlation of taxa and specimen weights/lengths	bone circumference types and element survival pattern, or body size classification for non- IDed.
	accumulator of deposit	%carnivore or fragmentation patterns used as proxies	direct observations of gnawing/digestion marks, element survival pattern (e.g., %MAU), %carnivore, taxonomic diversity, age profile
	preservation	ID rate linked to collagen preservation and deamidation level an additional proxy for individual bone preservation	weathering state, color, root etching, density-mediated attrition
	sex & mortality profiles	rare	morphometric measurements
	environment adaption or phylogenetic evolution	difficult to detect from COL1 amino acids mutations	morphometric data (large dataset required)
	anthropogenic modifications	difficult to observe if bones are too small (<2cm)	cut marks, burning, breakage patterns
	spatial distribution of fauna remains in deposit	when no exact coordinates are available, spatial distribution can be based on larger units (squares, layers)	If piece-plotted, bone will have exact spatial coordinates. When no exact coordinates are available, spatial distribution will be based on larger units (squares, layers).

It is difficult for the morphological approach unless using a large biometrics dataset for local species (Hanot and Bochaton 2018; Horsburgh 2018; Scott and Plug 2016).

ZooMS identification is not equally effective for all mammals. In ZooMS identification, a widely accepted reference system comprises 12 peptide markers—some are more detectable, others are more diagnostic. That is why ZooMS markers have different levels of effectiveness for identification. These discrepancies in identifiability are similar to those in morphological identification (Driver et al. 2011; Lyman 2015b; Wong et al. 2017).

Both ZooMS and morphological approaches may reach assignments broader than the genus. It is due to the inability

to locate or differentiate specific morphological features (Lau and Kansa 2018; Lyman 2015b), or the lack of diagnostic peptide markers in ZooMS spectra. Hence, the term “taxon” is used as and is not restricted to genus/species (Lyman 1994).

An advantage of ZooMS over comparative zooarchaeology is the standardized method for data analyses. With ZooMS, bones can be identified “each on its own merits” without many assumptions imposed by the analysts. On the contrary, the quality of a morphological analysis depends more on the analyst’s experience. Zooarchaeologists have noticed the inter-analyst variability for decades (Domínguez-Rodrigo 2012; Gobalet 2001; Lau and Kansa

2018). While initiatives like the blind test and quality control in the morphological analysis were proposed (Driver et al. 2011; Wolverton 2013), zooarchaeologists rarely undertake these in practice.

Although not entirely absolute, a ZooMS identification depends much less on the analyst's experience. Most ZooMS analysts process spectra following a standardized workflow (Brown 2021), if not automatic programming (Gu and Buckley 2018). In addition, it is easy to clarify specific criteria used to identify one taxon over the other. Nevertheless, a recent double-blind experiment has verified that ZooMS and morphological identification yield statistically indistinguishable taxonomic profiles for the same archaeological faunal assemblage (Morin et al. 2023). Ultimately, ZooMS can contribute to quality control of morphological analysis as long as collagen preservation allows.

**Data interpretation.** Following taxonomic assignments, the resulting dataset forms the basis for interpreting specific questions linked to the regional ecology, paleoclimate and human subsistence patterns. Morphological identification relies on the identity of the skeletal elements. Thus, the taxonomic results naturally include elemental composition and morphometric information. In contrast, ZooMS identification depends on the presence of diagnostic collagen peptides, which can reflect the biomolecular preservation of samples (Wang et al. 2021). Element survival pattern is essential in reconstructing human subsistence and is statistically quantitative. The lack of elemental information disadvantages faunal assemblages sieved from the sediment, like the Vogelherd ZooMS assemblage. However, in a few recent integration attempts, ZooMS assemblages have incorporated specimens representing specific anatomical elements or having traces of modification, while lacking precise taxonomic identifications (Pothier-Bouchard et al. 2020; Ruebens et al. 2023; Silvestrini et al. 2022; Torres-Iglesias et al. 2024).

ZooMS greatly augments the number of specimens that can be assigned to taxa. Meanwhile, the weight (or length) of each single specimen could be routinely measured before ZooMS lab work due to the generally smaller size of specimens in ZooMS assemblage. Combining weight (or length) data and expanded taxa identifications, ZooMS can provide powerful insights into bone fragmentation, preservation, and spatial distribution. Integrated with zooarchaeological results, ZooMS allows for a more comprehensive archaeological interpretation.

New proteomic approaches like SPIN with higher taxonomic resolution offer hope to replace ZooMS, providing higher quality and more in-depth results to zooarchaeology (Rüther et al. 2022). Ultimately, the integration of proteomic approaches, environmental sediment DNA (e.g., Zavala et al. 2021) and traditional zooarchaeology on species abundance will transform how we understand the faunal record of an archaeological or paleontological site.

## CONCLUSIONS

In the new era of ample scientific research in archaeology, a central goal is the combination of all analytical tools to

represent and understand the past better. As often quoted, scientific interpretations are only probable reconstructions of reality, and usually simply empirical approximations, rather than the absolute reality of what once was (Bunge 1998).

In this study, we use the history of research on the fauna of Vogelherd as a case study for understanding the change of research focus in archaeological fauna remains. The application of ZooMS represents the most recent analytical tool used at Vogelherd, and this method would greatly augment the number of specimens that can now be identified to the taxa. Here, we compiled the data from all previous and current studies and compared the datasets, in an attempt to provide guidelines for integrating and interpreting zooarchaeological data gained from morphological and ZooMS approaches.

While our interpretations remain an “empirical approximation” of past ecology and human behavior, we hope that by combining and integrating such datasets within a concise framework, researchers in the coming years will significantly expand our understanding of the archaeological record in ways that remained out of reach before the advent of ZooMS.

## DATA AVAILABILITY

We have uploaded all ZooMS spectra and results files to Mendeleev data. (“Vogelherd ZooMS data,” [doi: 10.17632/9jp4jdzy7k.1](https://doi.org/10.17632/9jp4jdzy7k.1))

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# Special Issue: Integrating ZooMS and Zooarchaeology: Methodological Challenges and Interpretive Potentials

## Supplement 1: Integrating Morphological and ZooMS-Based Approaches to Zooarchaeology at Vogelherd Cave in Southwestern Germany

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### SUPPLEMENT 1

This supplement includes: supplementary material text, supplementary material figures S1–S2, and supplementary material references. ZooMS spectra files (in .mzml formats) and results are accessible on Mendeley data (“Vogelherd ZooMS data,” [doi: 10.17632/9jp4jdzy7k.1](https://doi.org/10.17632/9jp4jdzy7k.1)).

### ZOOMS SAMPLE PREPARATION AND DATA ANALYSIS

We sampled 276 bones by removing a 20mg chip from each, and these were originally treated using the Ambic protocol. Then 114 samples that fail to yield ideal spectra, together with an additional 11 samples, were performed with acid-insoluble protocol as below.

**AmBic protocol.** Each bone sample was covered in 100µL 50 mM ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ) at room temperature overnight, to clean and remove soluble contamination. The supernatant was discarded and an additional 100µL of 50 mM  $\text{NH}_4\text{HCO}_3$  was added in. Following incubation at 65°C for 1 h, the bone chips were frozen at -20°C and the supernatant was digested with 0.4µg trypsin at 37°C for 18 h. After that, 1µL 5% trifluoroacetic acid (TFA) was added to end the digestion. The resulting supernatant was concentrated and desalted using C18 ZipTips, then washed with 200µL 0.1% TFA and eluted with 50µL 50% ACN/ 0.1% TFA (*v/v*).

**Acid-insoluble protocol.** The bone chips were demineralized in 500µL 0.5 M HCl for 24–48 h at 4°C until the bone chips became spongy and stopped reacting. The acid supernatant was removed. The chips were then rinsed 3 times

using 0.5M  $\text{NH}_4\text{HCO}_3$  until a neutral pH was reached. The samples were incubated at 65 °C for 1 h, in 100µL of 50 mM  $\text{NH}_4\text{HCO}_3$ . After the incubation, 50µL supernatant was digested and desalted as above.

All tryptic extracts were diluted 10 times with 50% ACN/0.1% TFA (*v/v*), and mixed with an equal volume of  $\alpha$ -cyano-4-hydroxycinnamic acid solution (10mg/mL in 50% ACN/0.1% TFA (*v/v*)). 1.5µL of the mixture was spotted on a Bruker ground steel plate in triplicate. One blank was analyzed alongside every twenty-three samples as a negative control. Samples were measured using an Autoflex Speed LRF Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometer (Bruker).

Mass spectra files were processed using the mMass open software version 5.5.0 (Strohalm et al. 2010). ZooMS identification is done with an in-room tool. Previously published type I collagen peptide markers were used for the taxonomic identification of each sample (Buckley and Kansa 2011; Buckley et al. 2009; Janzen et al. 2021; Welker et al. 2016).

Statistical analysis was conducted in R (Team and Others 2013) and figures were produced using the package ggplot2 (Wickham 2016).

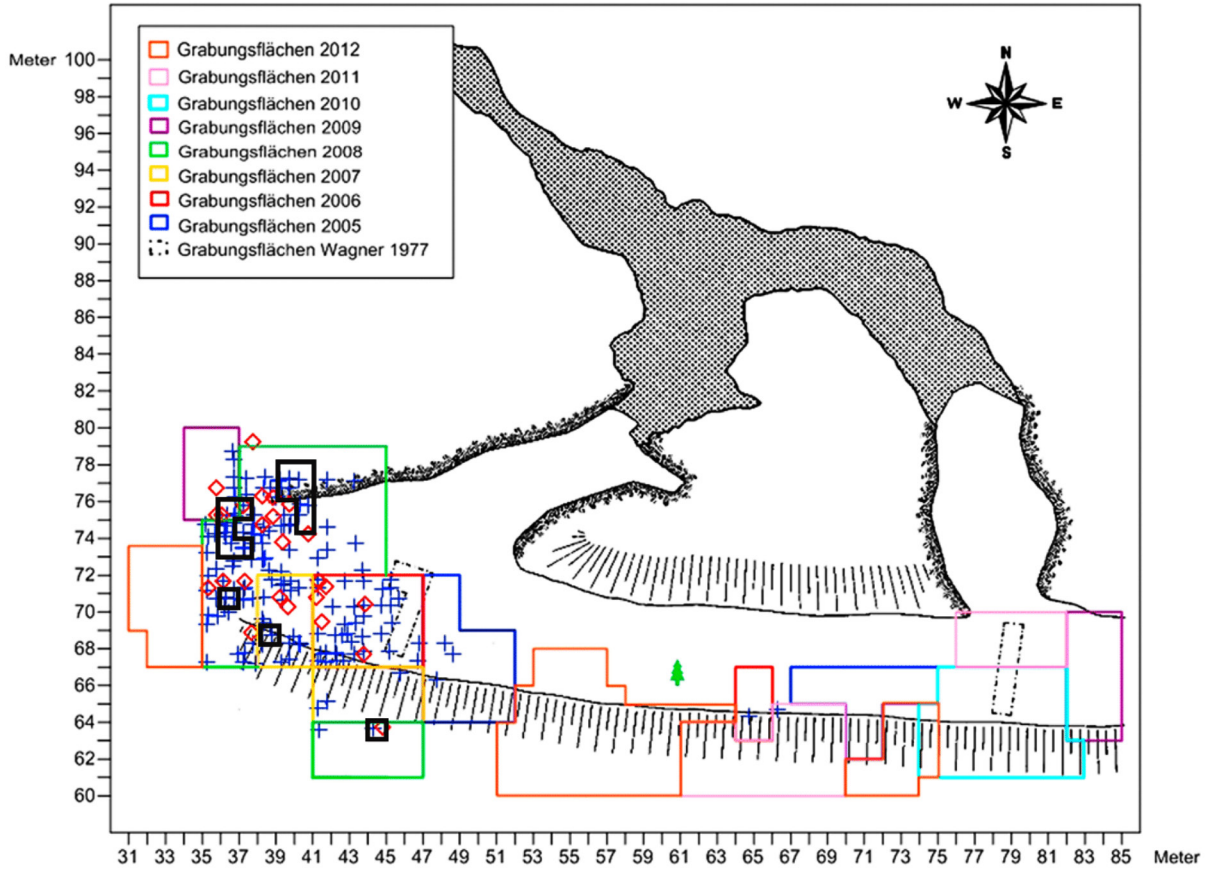


Figure S1. The plot of ZooMS sampled locations in black boxes (image modified from Wolf and Conard 2015). All double-perforated (blue cross) and single-perforated (red lozenge) beads were discovered in front of the southwest entrance, Vogelherd Cave.

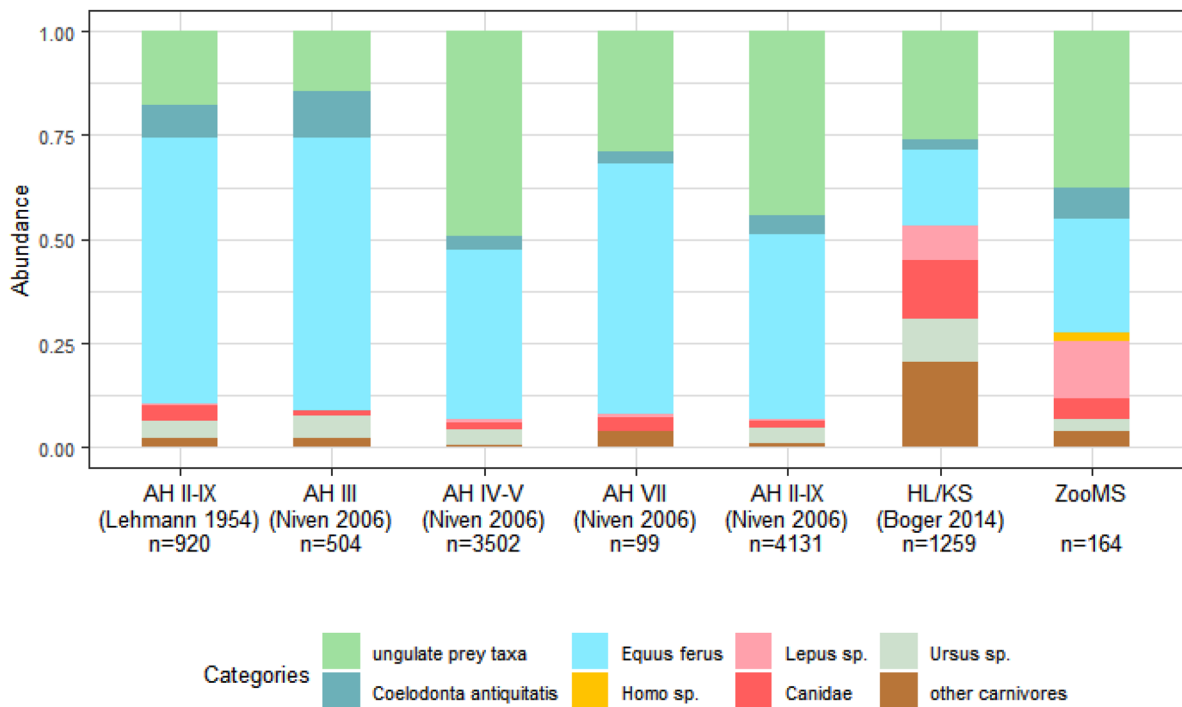


Figure S2. Abundance of identifiable specimens by taxonomic categories, using NISP values, Elephantidea excluded. Data from Boger et al. (2014), Lehmann (1954), and Niven (2006).

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