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

Can ZooMS Help Assess Species Abundance in Highly Fragmented Bone Assemblages? Integrating Morphological and Proteomic Identifications for the Calculation of an Adjusted ZooMS-eNISP

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ABSTRACT

Zooarchaeology by Mass Spectrometry (ZooMS) is a rapid, low-cost, collagen-based method for the taxonomic identification of animal tissues. It is now increasingly applied to bone fragments from archeological contexts, creating large taxonomic datasets. How to integrate these ZooMS identifications within general zooarchaeological theoretical frameworks, such as estimates of species abundance and taxonomic richness, remains problematic. Past large-scale ZooMS analyses of Eurasian Paleolithic faunal assemblages have shown a general trend towards an increased representation of large ungulates (mainly *Bos/Bison*) in the ZooMS fraction, often coupled with a decrease in medium-sized taxa (e.g. reindeer). Here we propose several hypotheses to explain these identification discrepancies, involving identification biases and differential fragmentation patterns across various taxa, and test them using the case study of the Paleolithic site of Cassenade.

At the Châtelperronian site of Cassenade (France), nearly all bone fragments larger than 20mm (n=1,119) have been identified to taxa, either through comparative morphology (n=364) or ZooMS (n=755). Each of these fragments was weighed and measured, creating a unique database to explore the relation between fragmentation and identification. Analysis shows that fragment size and mass distributions are distinct across taxa if only bones identified by morphology are considered, but, somehow counter-intuitively, extremely similar across taxa of various body sizes when all their bones are integrated. In particular, the bones of larger ungulates tend to be broken

into a higher number of fragments, inducing an over-representation of larger taxa in ZooMS-NISP. Our dataset also shows that sorting long-bone shaft fragments by body size classes should be seen by zooarchaeologists as a process that, in addition to being prone to risks of misidentification, provides highly biased information of little use for estimating species abundance. To overcome this issue, we propose the calculation of an adjusted equivalent ZooMS NISP (ZooMS-eNISP) by dividing, for each taxon, the total ZooMS mass of identified bones (g) by the mean mass of morphologically identified bones for that taxon (g/NISP). The advantage of this method is that it considers site-specific characteristics of the faunal assemblage, notably bone preservation, which is especially important in Paleolithic contexts. Finally, we propose that ZooMS-eNISP, despite its limits, can facilitate the integration of both identification methods to produce a more refined picture of patterns of species representation, site formation, and human behavior at an archaeological site.

INTRODUCTION

The last decade has seen considerable development in rapid, low-cost, collagen-based species identification methods (Buckley et al. 2009; Rüther et al. 2022; van Doorn et al. 2011; Welker et al. 2015). As a result, Zooarchaeology by Mass Spectrometry (ZooMS) is now increasingly applied to a range of archeological materials, including large-scale studies of bone fragments from Paleolithic contexts (Brown et al. 2016, 2021; Buckley et al. 2017; Deviese et al. 2017; Morin et al. 2023; Mylopotamitaki et al. 2024; Pothier Bouchard et al. 2020; Ruebens et al. 2022, 2023; Sinet-Mathiot et al. 2019, 2023; Welker et al. 2015, 2016, 2017). However, studies that integrate these large ZooMS datasets in the general framework of zooarchaeological interpretations are still in their infancy (Brown et al. 2021; Morin et al. 2023; Ruebens et al. 2023; Sinet-Mathiot et al. 2019, 2023; Smith et al. 2024; Torres-Iglesias et al. 2024). The actual impact of the newly acquired identifications of small morphologically unidentifiable bone fragments on our understanding of past subsistence strategies, prey selection, transport decisions, or butchering activities has yet to be fully uncovered.

Zooarchaeologists calculate species proportions typically through quantification of either the number of identified specimens (NISP) or minimum number of individuals (MNI) morphologically identifiable, the first being often favored for analysis of taxonomic abundance (Grayson 1984; Lyman 2018). Currently, large-scale ZooMS studies focus on the unidentifiable portion of the bone assemblage, both in terms of species and anatomical element, and hence often on smaller fragments. A key remaining question is the integration of ZooMS identifications with data obtained through “traditional” zooarchaeological studies in terms of understanding the abundance of different taxa—how can we best compare numbers of identified specimens obtained by two radically different methods that each have their own biases and are based on samples of, by research design, opposite characteristics? In some cases, ZooMS measures of abundance are at odds with the results previously obtained with morphological identifications (Table 1; Morin et al. 2023; Raymond et al. 2024 [this volume]; Ruebens et al. 2022, 2023; Sinet-Mathiot et al. 2019, 2023). In this contribution we explore one such discrepancy, the higher representation of large ungulates in ZooMS identifications, using the data acquired from the Paleolithic site of Cas-

senade (Discamps et al. 2019; Ruebens et al. 2024 [this volume]). We propose the calculation of an adjusted metric, an “equivalent NISP” for ZooMS identifications (ZooMS-eNISP), based on the mass of the bone fragments. This method allows us to achieve a more in-depth, site-specific integration of the morphological and ZooMS identifications, and a more representative interpretation of the abundance of the various identified animal taxa.

THE LARGE (UNGULATE) PROBLEM OF ZOO MS STUDIES

BACKGROUND

Previous large-scale ZooMS analyses of European Paleolithic faunal assemblages frequently highlighted a lower proportion of medium-sized taxa (e.g., reindeer) in the ZooMS fraction, coupled with an increased representation of large ungulates such as *Bos/Bison* (see Table 1; Morin et al. 2023; Raymond et al. 2024 [this volume]; Ruebens et al. 2023, 2024 [this volume]; Sinet-Mathiot et al. 2019, 2023). ZooMS sampling strategies might affect the resulting species representation by, for example, only including the largest bone fragments. However, in many of the large-scale studies cited above, all elements longer than 2cm or 3cm were analysed—this size cut-off is comparable to the one that would typically be used in a traditional zooarchaeological analysis of large mammals, hence sampling strategy can hardly be considered as the main factor behind the over-representation of large ungulates in ZooMS studies.

How then can we explain such a discrepancy in species abundance between ZooMS and morphological identifications? 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.

FRACTIONATION BIASES AND HYPOTHESES FOR EXPLAINING LARGE GAME OVER-REPRESENTATION

ZooMS sampling can be strongly impacted by zooarchaeologists’ practices—the way in which bones are identified morphologically, later sorted, and stored can easily produce a sampling bias. For example, if only the largest unidentifiable bones were subject to ZooMS analysis, the proportion

TABLE 1. THE PRESENCE OF *BOS/BISON* REMAINS IN SOME EURASIAN PALEOLITHIC ASSEMBLAGES (BG=Bulgaria, FR=France, DE=Germany, IT=Italy, RU=Russia), COMPARING THE NUMBER OF SPECIMENS IDENTIFIED BY MORPHOLOGY AND ZooMS.*

Site	Layer	NISP		<i>Bos/Bison</i> (%NISP)		Trend	Ref.
		Morpho.	ZooMS	Morpho.	ZooMS		
Abri du Maras (FR)	1	49	226	18%	30%	x1.67	4
Bacho Kiro (BG)	I	1 077	776	26%	46%	x1.77	8
Bacho Kiro (BG)	J	232	433	9%	27%	x3.00	8
Bacho Kiro (BG)	K	143	337	27%	33%	x1.22	8
Cassenade (FR)	2	698	838	17%	51%	x3.00	6
Denisova (RU)	East chamber	5339	5161	9%	32%	x3.55	1
Denisova (RU)	Main chamber	554	905	12%	36%	x3.00	1
Denisova (RU)	South chamber	1894	579	10%	38%	x3.80	1
Fumane (IT)	A3	453	222	7%	41%	x5.86	7
Fumane (IT)	A4	681	275	5%	30%	x6.00	7
Les Cottés (FR)	4 upper	630	70	4%	7%	x1.75	8
Les Cottés (FR)	4 lower	715	168	7%	18%	x2.57	8
Les Cottés (FR)	6	166	217	22%	40%	x1.82	8
Les Cottés (FR)	8	397	220	42%	74%	x1.77	8
La Ferrassie (FR)	6	142	457	6%	50%	x8.33	8
Le Piage (FR)	Early Auri.	117	117	6%	8.5%	x1.42	2
Le Piage (FR)	GI	2549	744	6%	10.60%	x1.77	3
Salzgitter (DE)	all	5426	584	1%	3%	x3.00	5

*Only large-scale ZooMS studies (>100 samples) with high ZooMS success rates (>70%) are included. The trend relates to the multifold increase. References (ref.): 1) Brown et al. 2021, 2) Morin et al. 2023, 3) Raymond et al. 2024 (this volume), 4) Ruebens et al. 2022, 5) Ruebens et al. 2023, 6) Ruebens et al. 2024 (this volume), 7) Sinet-Mathiot et al. 2019, 8) Sinet-Mathiot et al. 2023.

of larger ungulates would likely increase, in a comparable fashion to what is produced by biased recovery methods of faunal material (Discamps and Faivre 2017). However, in many cases such as at Cassenade, the discrepancy between ZooMS and morphological species abundance can-

not simply be explained by a sampling effect, considering that nearly all morphologically unidentifiable plotted bones larger than 2cm were sampled for ZooMS (Ruebens et al. 2024 [this volume]). Thus, other mechanisms must be considered.

Number of identified specimens based on morphological or ZooMS identification (abbreviated in the following morpho-NISP and ZooMS-NISP, respectively) are both strongly affected by bone fragmentation. For example, increased bone breakage will produce smaller fragments that are more difficult to identify morphologically. Further, differential identification phenomena would likely come into play—at comparable degrees of fragmentation, some skeletal elements are more difficult to identify (e.g., cranial, axial elements, as shown by Morin et al. 2023), as well as some species (i.e., zooarchaeologists would be less confident in identifying rare species such as ibex or saiga if the material is highly fragmented). These differential identification biases on morpho-NISPs are often suspected by faunal specialists, but still poorly described and quantified, and they are probably highly observer-dependent (see, for example, Morin et al. 2017). While ZooMS is well placed to test and address some of these issues, it is also impacted by some of the same issues—because ZooMS analysts sample what has been considered “unidentifiable” by zooarchaeologists, the differential identification biases of morpho-NISPs may simply be mirrored in the ZooMS-NISPs. Hence, bone fragmentation has the potential to have a dramatic impact on abundance measurements derived from ZooMS data, through the influence of zooarchaeologists’ practices.

To explain the over-representation of larger ungulates, several hypotheses linked to bone fragmentation can be proposed (Figure 1). Obviously, these hypotheses are neither exclusive nor comprehensive.

Hypothesis 1 - All Taxa Produce Similar Quantities of Bone Fragments

In hypothesis 1, fragmentation produces a similar number of fragments per bone for each taxa (and thus, consequently, bigger fragments for larger ungulates)—bone fragment size is scaled with the size of the species. A high rate of fragmentation could considerably increase differential identification bias for species of different body size. In many Paleolithic faunal assemblages, larger ungulates include a higher number of species than medium sized ones. Typically, if a faunal assemblage is composed of horse, large bovid, and reindeer bones, reindeer fragments would theoretically be easier to distinguish morphologically because of their smaller cortical size. In hypothesis 1, bone fragmentation would increase this differential identification bias, making it harder to distinguish horse from large bovid remains and artificially increasing the proportion of reindeer based on morpho-NISPs. ZooMS-NISPs would mirror this bias, with an over-representation of large ungulates in the ZooMS sample. In that case, integrating ZooMS would provide a much stronger measurement of species abundance. This hypothesis is often put forward to explain discrepancies in taxonomic abundances (e.g., Sinet-Mathiot et al. 2023).

Hypothesis 2 - Larger Taxa Produce More Bone Fragments

Bone fragmentation may produce fragments with a similar size distribution independent of taxa. Thus, taxa with

larger complete bones would be broken in more fragments. For larger ungulates, a large quantity of “relatively small fragments,” as relative to the size of complete bones, would be produced. This would not necessarily affect morpho-NISP, as morphological identifications are not linked to the absolute size of bone fragments, but rather to the relative size of them (i.e., chances of preserving an anatomical feature such as a muscular insertion, foramen, crest, etc.). Such a scenario might happen if bone fragmentation is mostly governed by the physical properties of the bones and the physical constraints involved in bone breakage (i.e., forces applied to the bones). A similar granulometry (size distribution of fragments) would be produced independent of taxa (see Figure 1), in a similar fashion as what is known for flint knapping (Bertran et al. 2012). In this hypothesis, breaking large ungulates bones would produce more fragments, inducing an over-representation of them in ZooMS-NISPs. Integrating ZooMS-NISPs to the morpho-NISPs counts would provide a worse measurement of species abundance.

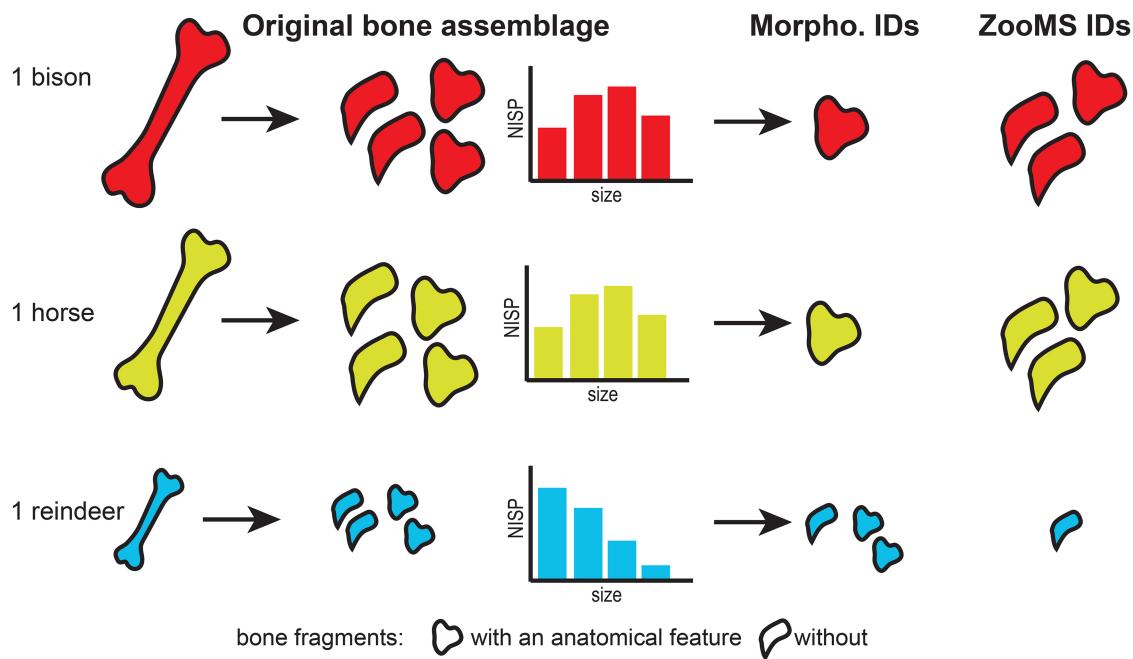
Many other factors might come into play (cf. Discussion section), but we here try to test these two hypotheses using the Cassenade dataset.

CASE STUDY: CASSENADE

MATERIAL AND METHODS

Cassenade is a Paleolithic site located in southwestern France, in the municipality of Saint-Martin-des-Combes (Dordogne). It was the focus of recent (2012–2013) excavations of deposits corresponding to the filling of a collapsed karstic corridor. Numerous faunal remains (>2,000) representing both human and carnivore occupations were recovered at the site, as well as 212 lithic artifacts attributable to the Châtelperronian (Discamps et al. 2019). The faunal material, mostly accumulated by cave hyenas, became intermingled by surface water runoff with evidence of a small hominid stop-over occupation and a cave bear den. While the site was previously divided into two assemblages, they are here combined for the sake of our analysis that is focused on the general pattern of fragmentation at the site, considering that the abundance of each taxa is relatively similar between assemblages (with the exception of ursids that are more frequent in the lower assemblage) and the low sample size (n=41) of morphologically identified bones for the lower assemblage.

Considering that our aim is to compare ZooMS and morphological identifications, ZooMS identifications that were taxonomically less precise were excluded (Bovidae/Cervidae, Canidae, Cervid/*Saiga/Capreolus*, Hyaenidae/Panthera, Ursidae/Felinae), as well as small vertebrates (bats, birds, rodents, lagomorphs) and taxa that were only identified by ZooMS (*Panthera* sp.) or by morphology (*Capreolus capreolus*, *Vulpinae*). In order to simplify analyses, equids were grouped (morphological identifications identified 150 *Equus caballus* remains for only 5 *Equus hyaenoides*), as well as large cervids (morphological identifications identified 6 *Cervus elaphus* remains and 3 *Megaceros giganteus*).

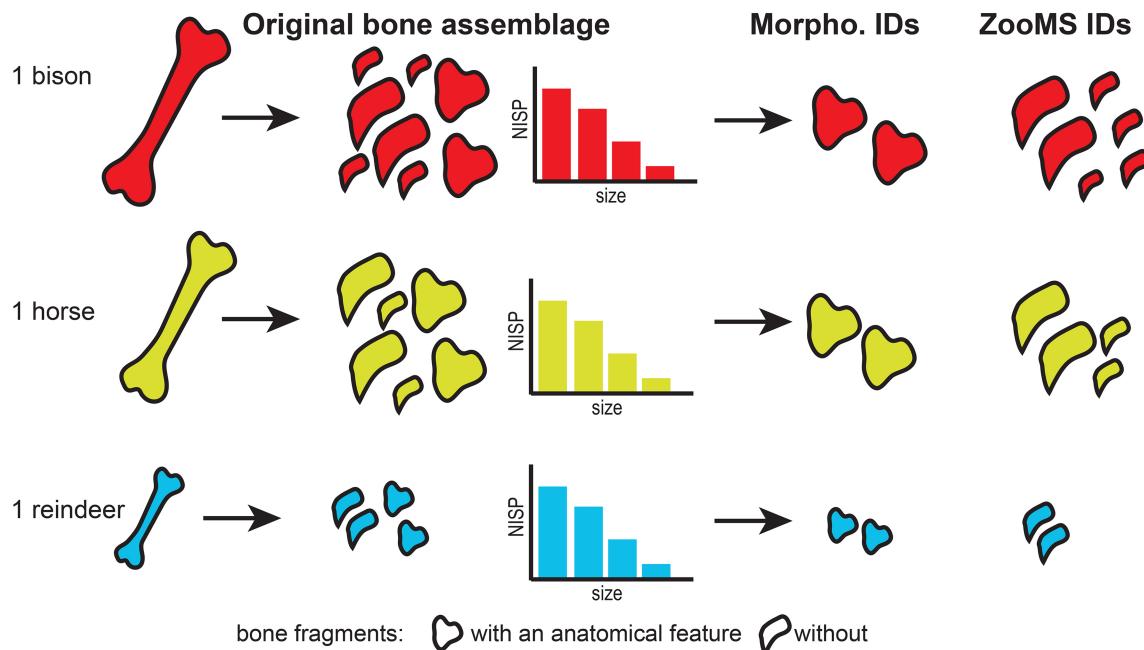
Hypothesis 1: all taxa produce similar quantities of bone fragments

Bison and horse fragments are difficult to distinguish from each other, lowering their identification rates.

Reindeer fragments are easier to distinguish because of their smaller cortical size.

Higher fragmentation increases this differential identification bias.

Integrating ZooMS NISP provides a better measurement of species relative abundance.

Hypothesis 2: larger taxa produce more bone fragments

Fragmentation produces bone fragments of similar absolute sizes for each taxa (similar distributions).

Thus, taxa with bigger complete bones are broken in more fragments per bone.

Identification success mostly depends on the presence of an anatomical feature, irrespective of fragment size.

Integrating ZooMS NISP provides a worse measurement of species relative abundance.

Figure 1. Two hypotheses linked to bone fragmentation that could explain the over-representation of large ungulates in ZooMS-NISP.

TABLE 2. TOTAL MORPHO-NISP AND ZooMS-NISP BY ASSEMBLAGE AT CASSENADE, INCLUDING TEETH AND SMALL BONES THAT WERE NOT INCLUDED FOR FURTHER ANALYSIS*.

Body size class	Taxon	Lower assemblage			Upper assemblage		
		morpho bone	morpho teeth	ZooMS	morpho bone	morpho teeth	ZooMS
Medium	CROC	4	11	5	14	18	9
	RANG	5	1	2	8	1	17
	SUS	-	2	1	-	-	1
Medium - Large	CELMEG	2	1	12	4	2	27
	EQUID	2	29	26	26	77	131
	URS	9	48	43	1	6	10
Large	BB	18	10	90	32	22	303
Large - very large	RHINO	1	5	18	1	6	39
Very large	PROBO	-	-	3	-	1	18
Total		41	107	200	86	133	555

*Abbreviations used for each taxa throughout the manuscript are as follows: BB=aurochs & bison, CELMEG=red deer & megaloceros, CROC=hyena, EQUID=horse & wild ass, PROBO=mammoth, RANG=reindeer, RHINO=rhinoceros, SUS=wild boar, URS=bear.

teus teeth), proboscideans (all probably corresponding to *Mammuthus primigenius*, but with no definitive evidence) and rhinoceros (morphological study identified only *Coelodonta antiquitatis*).

Thus, here we consider 9 taxa from Cassenade: *Bos/Bison* (BB), *Cervus elaphus/Megaloceros giganteus* (CELMEG), *Crocuta crocuta spelaea* (CROC), equids (EQUID), *Mammuthus primigenius* (PROBO), *Rangifer tarandus* (RANG), *Coelodonta antiquitatis* (RHINO), *Sus scrofa* (SUS) and *Ursus spelaeus* (URS). Table 2 and Figure 2 present the proportions of these 9 taxa with both morphological and ZooMS identifications. Cranial/postcranial proportions vary widely according to taxa, but this pattern cannot be investigated with the dataset at hand—indeed, most of the unidentifiable tooth fragments were not sampled for ZooMS analysis (99% of ZooMS identifications were performed on bones; 750/755). Thus, we decided to focus this study on bone material, and only bone fragments longer than 2cm were included for further analysis. A total of 121 morpho-NISP and 742 ZooMS-NISP were included in the study (Table 3). When morpho-NISP and ZooMS-NISP are merged (see Table 3), the proportions of some larger mammals increases

(Bovinae +11%, proboscideans +2%, and rhinoceros +5%), while the proportions from medium-sized mammals decreases (cave hyena -9%, reindeer -7%).

For this contribution, data acquired by morphological identifications (Discamps et al. 2019) and ZooMS analysis (Ruebens et al. 2024 [this volume]) were combined in a single datasheet (available in Supplementary Material SI#1: <https://doi.org/10.5281/zenodo.12600092>). Each bone fragment was measured by 1cm size classes (using concentric circles drawn on a piece of paper) and weighed to the nearest centigram (using a Sartorius Entris 3200i-1S balance). All the data processing and statistical analysis were performed using R Statistical Software (v4.2.2; R Core Team 2021; RStudio Team 2020), and the code is available in Supplementary Material SI#2: <https://doi.org/10.5281/zenodo.12600092>.

SIZE AND WEIGHT DISTRIBUTIONS OF BONE FRAGMENTS

The Cassenade dataset offers a rare opportunity—nearly all the bone fragments that are >2cm were identified taxonomically, either by morphology or ZooMS. It is thus possible to

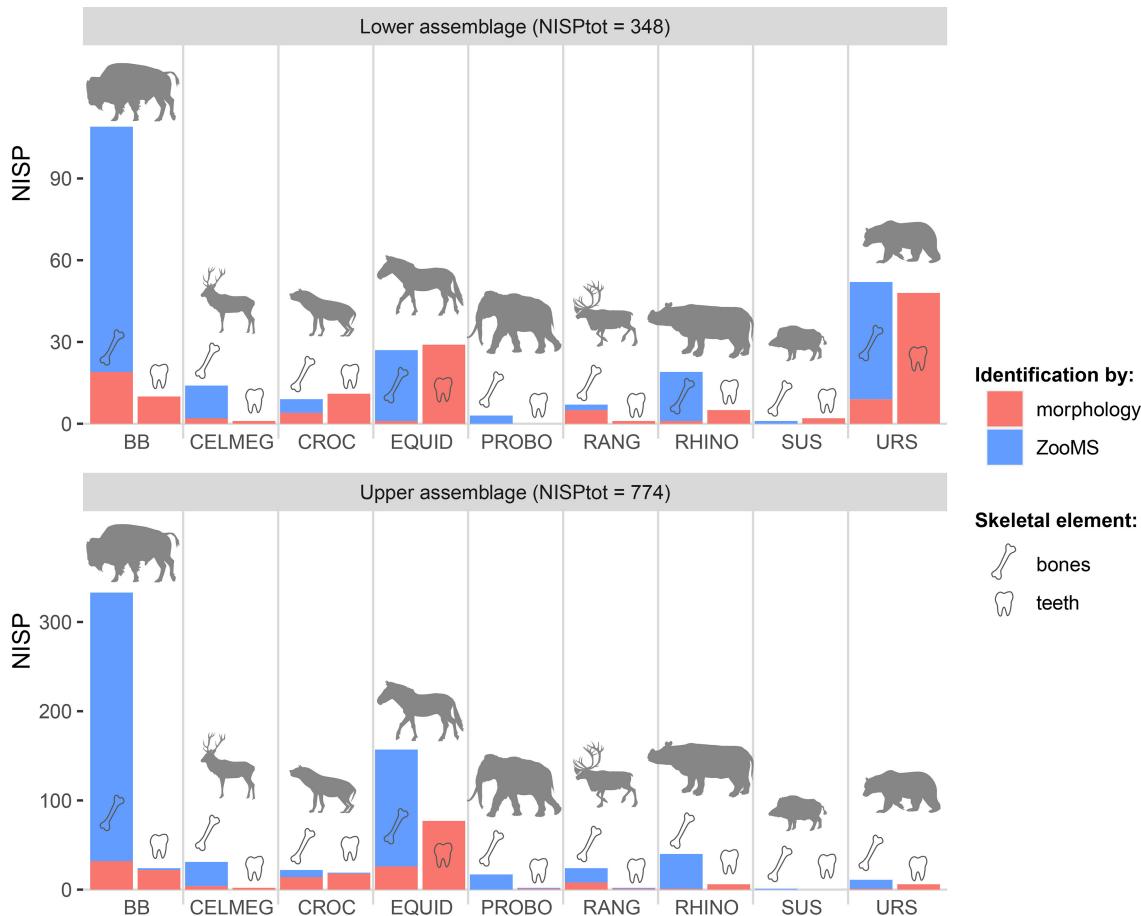


Figure 2. General overview of the number of identified remains (NISP) by morphology and ZooMS at Cassenade for the nine taxa selected for this study. (See Table 2 caption for reference to the abbreviations used for each taxon.)

test the hypotheses put forward in Figure 1 by studying the size and mass distribution of all bone fragments. Figures 3 and 4 present, respectively, the size and mass distributions of bone fragments for the eight main taxa present at Casenade (*Sus scrofa*, with only two identified bone remains, was excluded from the following).

Fragmentation produces a majority of small (less than 5cm) and light (less than 3g) fragments for all species, with relatively similar size and mass distributions. Our results corroborate hypothesis 2 (see Figure 1) and suggests, somewhat counterintuitively, that despite the large differences in scales of elements from large bovids, rhinoceroses, and reindeer, their breakage patterns yield remarkably similar granulometry.

In detail, four different patterns of fragmentation can be distinguished: 1) Bovinae, equids, rhinoceroses, and proboscideans (see Figure 3A to 3D) have extremely similar size distribution; 2) large deer and reindeer (see Figure 3E and 3F) are less fragmented, with size distributions that are slightly skewed towards larger bones (with more bones between 4cm and 5cm); 3) bear are closer to the second group, but with slightly more bones of 2–3cm (a pattern that is probably explained by the abundance of bear cubs at the site, cf. Discamps et al. 2019); and, 4) hyena bones are much

less fragmented. In 7 out of 8 taxa, mean sizes are very close and median sizes are identical (in agreement with hypothesis 2); hyena bones are less fragmented, an observation that can easily be understood by a different taphonomic history. Processes of fragmentation (diagenesis, hyena bone breakage, hominid marrow extraction, etc.) probably impacted bone size distribution differently for some species, such as cave hyenas. Median masses are very similar for most taxa—on average, for each taxon, about 50% of the bones are less than 2g, including for very large taxa such as rhinoceroses.

Interestingly, Figures 3 and 4 also show that the proportion of morphologically identified reindeer is considerably more important for small bones (size between 3cm to 5cm, mass between 1g to 5g) compared to other taxa. Size and mass distributions of morphologically identified specimens (Table 4) illustrate well the fact that morphological identification success is not linked to absolute size, but rather to relative size (in comparison to the complete bone), with larger size and mass for larger taxa. The size and mass of morphologically identified fragments vary widely between taxa, for example, Bovinae and reindeer morphologically identified bones have, respectively, mean sizes of 10cm and 5.7cm, and mean masses of 41.7g and 8g.

TABLE 3. SAMPLES SELECTED FOR FURTHER ANALYSIS IN THE PRESENT STUDY
 (these include only bone fragments longer than 2cm identified by morphology or ZooMS
 [lower and upper assemblages are merged, teeth are excluded])*.

Body size class	Taxon	Morpho NISP	ZooMS NISP	Total NISP	%NISP Morpho.	%NISP ZooMS	%NISP Total
Medium	CROC	15	13	28	12%	2%**	3%**
	RANG	13	18	31	11%	2%**	4%**
	SUS	0	2	2	0%	0%	0%
Medium - Large	CELMEG	6	39	45	5%	5%	5%
	EQUID	27	155	182	22%	21%	21%
	URS	9	52	61	7%	7%	7%
Large	BB	49	387	436	40%	52%*	51%*
Large - very large	RHINO	2	57	59	2%	8%*	7%*
Very large	PROBO	0	19	19	0%	3%	2%
Total		121	742	863			

Chi-square tests highlight cases for which the difference with %NISP established by morphological identifications is statistically significant (for p<0.05, ** for p<0.01). See Table 2 caption for reference to the abbreviations used for each taxon.

Kolmogorov-Smirnov statistical tests on size distributions confirm the above observations—when all bones are considered, the size distribution is similar for all taxa, except hyena (Table 5), while the size distribution of morphologically identified bones is distinct for most taxa (Table 6).

All in all, it is more likely for larger ungulates to have their bones broken in pieces that are too small to be identified, compared to smaller taxa. Thus, ZooMS studies of unidentified small bones would have the tendency of artificially increasing the proportion of larger ungulates. Table 4 shows that, despite the fact that only a very small number of the bone fragments could be morphologically identified, the total mass they represent is often much more important than ZooMS fragments. For example, if only 11% of the Bovinae fragments could be morphologically identified, these 48 remains (out of 435) represent 67% of the total mass of material attributable to the taxon. For reindeer, 42% of the remains were identified, yet the mass proportion is relatively similar to Bovinae, with 75% of the total mass of reindeer bone material identified by morphology. In sum, Table 4 shows that, even if, due to the characteristics of bone breakage, there is a much lower chance of being able to successfully identify morphologically large ungulate

fragments, the total amount of material that is identified is still comparable between species. This shows that ZooMS-NISPs can be a poor measure of relative species abundance when mass is not considered, as in general ZooMS-NISPs only represent a small proportion of the total mass of a bone assemblage. Importantly, these data contradict the expectations zooarchaeologists and proteomic specialists might have, as, for example, with the hypotheses suggested by Sinet-Mathiot et al. (2023: 15), or in hypothesis 1 (see Figure 1).

CALCULATING AN ADJUSTED ZooMS-eNISP

Making species abundance measurements more reliable requires designing units of quantification that are not affected by differences between species in the number of fragments produced by breakage for each bone. Morpho-NISPs are, by their nature and their dependence on “relative” size, less affected by this bias. For ZooMS-NISP, we propose a method to compute a “corrected” equivalent ZooMS-NISP (ZooMS-eNISP), by dividing, for each taxa, the total ZooMS mass of identified bones (g) by the mean mass of morphologically identified bones (g/NISP). In essence, this estimate is trying to answer the following question: “if one was able to

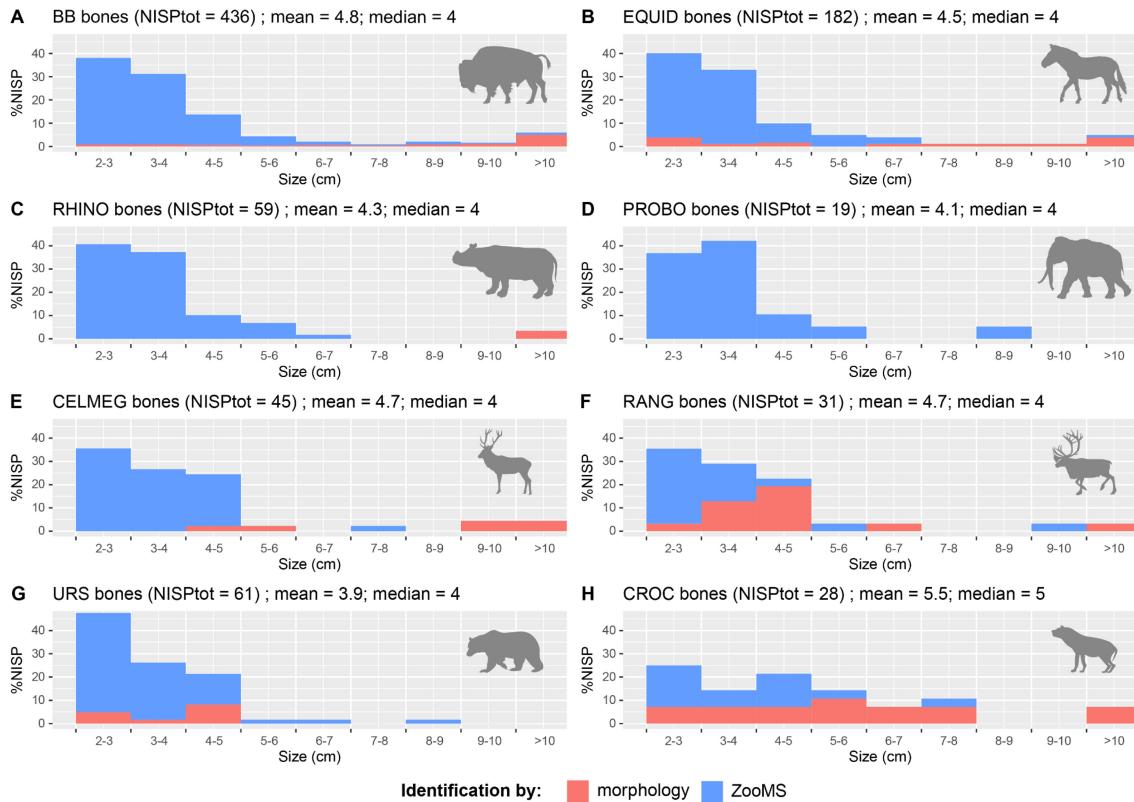


Figure 3. Size distribution of bone fragments identified by morphology (red) and ZooMS (blue). (See Table 2 caption for reference to the abbreviations used for each taxon.)

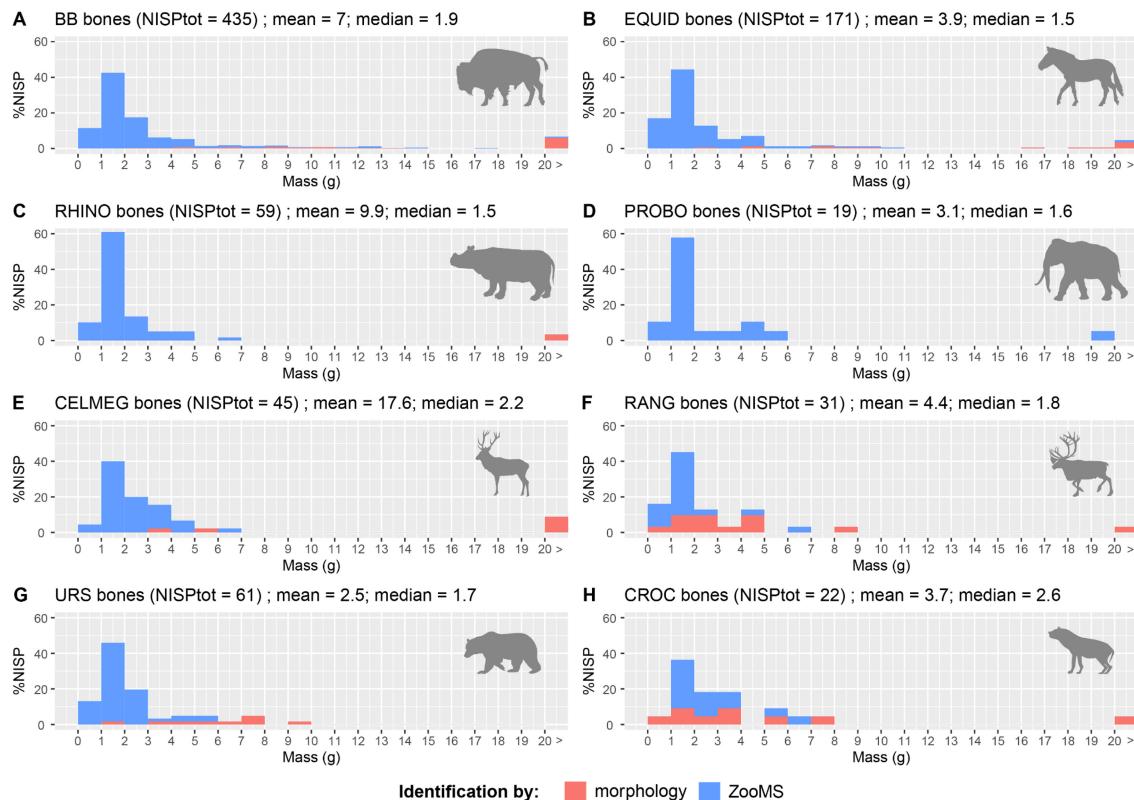


Figure 4. Mass distribution of bone fragments identified by morphology (red) and ZooMS (blue). (See Table 2 caption for reference to the abbreviations used for each taxon.)

TABLE 4. NISP, MASS MEANS AND MEDIANs FOR ALL BONE FRAGMENTS, AND FOR ONLY MORPHOLOGICAL IDENTIFICATIONS (the two last columns calculate the proportion of morphologically identified material according to NISP or to mass)*.

Taxon	All bone fragments				Morphologically identified				Proportions of morpho. IDs	
	NISP*	Total mass (g)	Mean mass (g)	Median mass (g)	NISP*	Total mass (g)	Mean mass (g)	Median mass (g)	% NISP	% mass
BB	435	3030.10	6.97	1.86	48	2035.72	42.41	24.31	11.0%	67.2%
CELMEG	45	793.59	17.64	2.17	6	706.33	117.72	105.07	13.3%	89.0%
CROC	22	82.28	3.74	2.58	9	47.60	5.29	3.07	40.9%	57.9%
EQUID	171	675.23	3.95	1.50	16	296.11	18.51	17.13	9.4%	43.9%
PROBO	19	58.29	3.07	1.59	0	0	0	0	0%	0%
RANG	31	137.73	4.44	1.78	13	103.89	7.99	2.81	41.9%	75.4%
RHINO	59	582.93	9.88	1.50	2	479.24	239.62	239.62	3.4%	82.2%
SUS	2	2.41	1.21	1.21	0	0	0	0	0%	0%
URS	61	154.61	2.53	1.69	9	55.71	6.19	6.93	14.8%	36.0%
Total	845	5517.17			103	3724.60				

*NISP values may not correspond to the ones reported in Table 3 as some bones could not be measured or weighed. See Table 2 caption for reference to the abbreviations used for each taxon.

refit small fragments identified by ZooMS to fragments of a size that would make them morphologically identifiable, how many refitted fragments would that be?"

Applying this calculation to the Cassenade dataset results in slightly different species abundances (Table 7, Figure 5), which are closer to proportions obtained by morphology only, yet still different. ZooMS-eNISP evens out the over-representation of Bovinae and rhinoceros, as well as the under-representation of reindeer and hyenas.

DISCUSSION

Analysis of size and mass distributions of Cassenade bones demonstrate that using ZooMS-NISP as a proxy for species abundance is not without problems, hence the proposition of a "corrected" ZooMS-eNISP based on the mean mass of morphologically identified bones in the assemblage. This metric is not without its own limits, most notably its heavy reliance on morphological identifications for its calculation, meaning that ZooMS-eNISP is by nature partly affected by

all the same biases (e.g., differential identification between species).

HOW BEST TO CORRECT ZooMS NISPs

Another possibility to "correct" ZooMS NISPs would be to use some other metrics, for example, by dividing ZooMS masses by the average mass of complete bones (Saunders 2023), or by measuring bone surface areas (as in Discamps 2011a). Adding up the total mass of identified fragments (by morphology or ZooMS) for a taxon and dividing it by the mass of a complete skeleton would allow for the calculation of some sort of "minimal number of skeletons." However, each method has advantages and drawbacks.

For ZooMS-eNISP, using the average mass of morphological identifications, a site-specific measure, has the following advantages: a) contrary to a method that would use masses from reference skeletons, there is no need to establish an *a priori* on past skeletal-part distribution or age composition (a problem in ZooMS-NISP normalization that was

TABLE 5. KOLMOGOROV-SMIRNOV STATISTICAL TESTS ON SIZE DISTRIBUTIONS, WHEN BOTH MORPHOLOGICAL AND ZooMS IDENTIFICATIONS ARE INCLUDED*.

TAXON	BB	CELMEG	CROC	EQUID	PROBO	RANG	RHINO	SUS	URS
BB		0.987	0.018	0.992	0.799	1.000	0.826	0.240	0.418
CELMEG	0.070		0.059	0.350	0.387	0.990	0.174	0.267	0.330
CROC	0.300	0.260		0.001	0.020	0.079	0.001	0.131	0.005
EQUID	0.038	0.109	0.338		0.899	0.662	0.981	0.235	0.514
PROBO	0.097	0.167	0.397	0.068		0.630	0.993	0.200	0.648
RANG	0.047	0.047	0.264	0.086	0.144		0.425	0.252	0.491
RHINO	0.087	0.157	0.387	0.070	0.038	0.134		0.220	0.655
SUS	0.619	0.644	0.750	0.599	0.632	0.645	0.593		0.300
URS	0.121	0.120	0.345	0.121	0.107	0.121	0.069	0.525	

*Upper diagonal reports p values (bold numbers: p<0.05), lower diagonal reports the D statistic. See Table 2 caption for reference to the abbreviations used for each taxon.

TABLE 6. KOLMOGOROV-SMIRNOV STATISTICAL TESTS ON SIZE DISTRIBUTIONS, WHEN ONLY MORPHOLOGICAL IDENTIFICATIONS ARE INCLUDED*.

TAXON	BB	CELMEG	CROC	EQUID	PROBO	RANG	RHINO	SUS	URS
BB		0.972	0.002	0.086	NA	0.000	0.044	NA	0.000
CELMEG	0.163		0.073	0.515	NA	0.010	0.071	NA	0.002
CROC	0.499	0.533		0.262	NA	0.044	0.015	NA	0.008
EQUID	0.269	0.333	0.274		NA	0.036	0.002	NA	0.014
PROBO	NA	NA	NA	NA		NA	NA	NA	NA
RANG	0.622	0.679	0.446	0.405	NA		0.029	NA	0.385
RHINO	0.878	1.000	1.000	1.000	NA	0.923		NA	0.018
SUS	NA	NA	NA	NA	NA	NA	NA		NA
URS	0.776	0.833	0.600	0.556	NA	0.256	1.000	NA	

*Upper diagonal reports p values (bold numbers: p<0.05), lower diagonal reports the D statistic. See Table 2 caption for reference to the abbreviations used for each taxon.

TABLE 7. ZooMS-eNISP CALCULATIONS FOR CASSENADE DATASET⁺.

Taxon	Morpho NISP* (bones)	Morpho g/NISP (bones)	ZooMS NISP (bones)	ZooMS total mass (g)	ZooMS eNISP	Morpho-NISP (%)	Morpho-NISP + ZooMS-NISP (%)	Morpho-NISP + ZooMS-eNISP (%)
BB	48	42.41	387	994.38	23.45	46.6%	52.8%	40.9%
CELMEG	6	117.72	39	87.26	0.74	5.8%	5.5%	3.9%
CROC	9	5.29	13	34.68	6.56	8.7%	2.7%	8.9%
EQUID	16	18.51	155	379.12	20.49	15.5%	20.8%	20.9%
RANG	13	7.99	18	33.84	4.23	12.6%	3.8%	9.9%
RHINO	2	239.62	57	103.69	0.43	1.9%	7.2%	1.4%
URS	9	6.19	52	98.90	15.98	8.7%	7.4%	14.3%
Total	103		721	1731.87	71.87			

*Includes only bones that could be weighted. See Table 2 caption for reference to the abbreviations used for each taxon.

also noted by Brown et al. 2021), as it directly “mimics” the ones reconstructed from morphological identifications; b) it bypasses the problem of taphonomic alterations of bone mass; and, c) it is not necessary to weigh modern skeletons from large reference collections. Disadvantages of ZooMS-eNISP include: a) the incapacity of including species that are identified only by ZooMS, or only by teeth (this is the case at Cassenade for mammoth, *Panthera* sp., and wild boar); b) the likely decrease in the importance of rare taxa (zooarchaeologists have a tendency to identify new species only if they are sure, hence the mean mass of morphological identifications would be higher); and, c) the necessity to weigh all the bones morphologically identified. Despite disadvantages, obtaining masses for a collection of modern skeletons would make it possible to compare mass ratios between skeletons of two species and, hence, test hypotheses on the differential fragmentation of these taxa (e.g., for a similar number of Bovinae and reindeer skeletons accumulated at a given site, how much more bone mass should we expect for Bovinae?).

PUSHING ZooMS-eNISP FURTHER

Refining ZooMS-eNISP would require more comparison, including mass data of morphologically identified bones from other sites, and the analysis of size distribution of other bone assemblages. For example, obtaining mass data on many different assemblages should make it possible to obtain average values for morphologically identified bones of rare taxa, and thus make it possible to calculate ZooMS-eNISP even if a species was not identified by morphology, or if masses could not be measured on morphological iden-

tified bones. ZooMS-eNISP was designed as a site-specific measurement due to biases affecting weights (such as taphonomic factors), yet obtaining average g/NISP values from many different sites might help in getting a better idea of the range of expected variation between different species (e.g., are Bovinae g/NISP values typically about 5 times higher than reindeer g/NISP ones?). ZooMS-eNISP has the default of an over-reliance on the characteristics of morphological identifications at a given site, hence further studies analyzing size and mass distributions would be of great help.

Apart from the two hypotheses proposed in Figure 1, bone granulometry induced by fragmentation might be complicated by other factors, such as differential fragmentation induced by species-specific differences in bone structure (e.g., more spongy bone in horse long bones compared to large bovid ones), physical constraints of bone breakage that would produce more splintering for larger and stronger bones (i.e., when the forces applied to the bones are insufficient to produce an adequate breakage), differential carcass processing by humans (e.g., bones that were not, or less, broken for marrow extraction), or, simply a different taphonomic history (e.g., bones that were accumulated by a different agent or bone granulometry affected by site formation processes). This is notably important if we want to understand why horse bones do not seem to be affected by the same overrepresentation in ZooMS-NISPs contrary to other larger ungulates (at least at Cassenade and Denisova Cave, cf. Brown et al. 2021). Many such hypotheses have still to be tested, as the Cassenade dataset has its limitations—the sample size is small for several taxa,

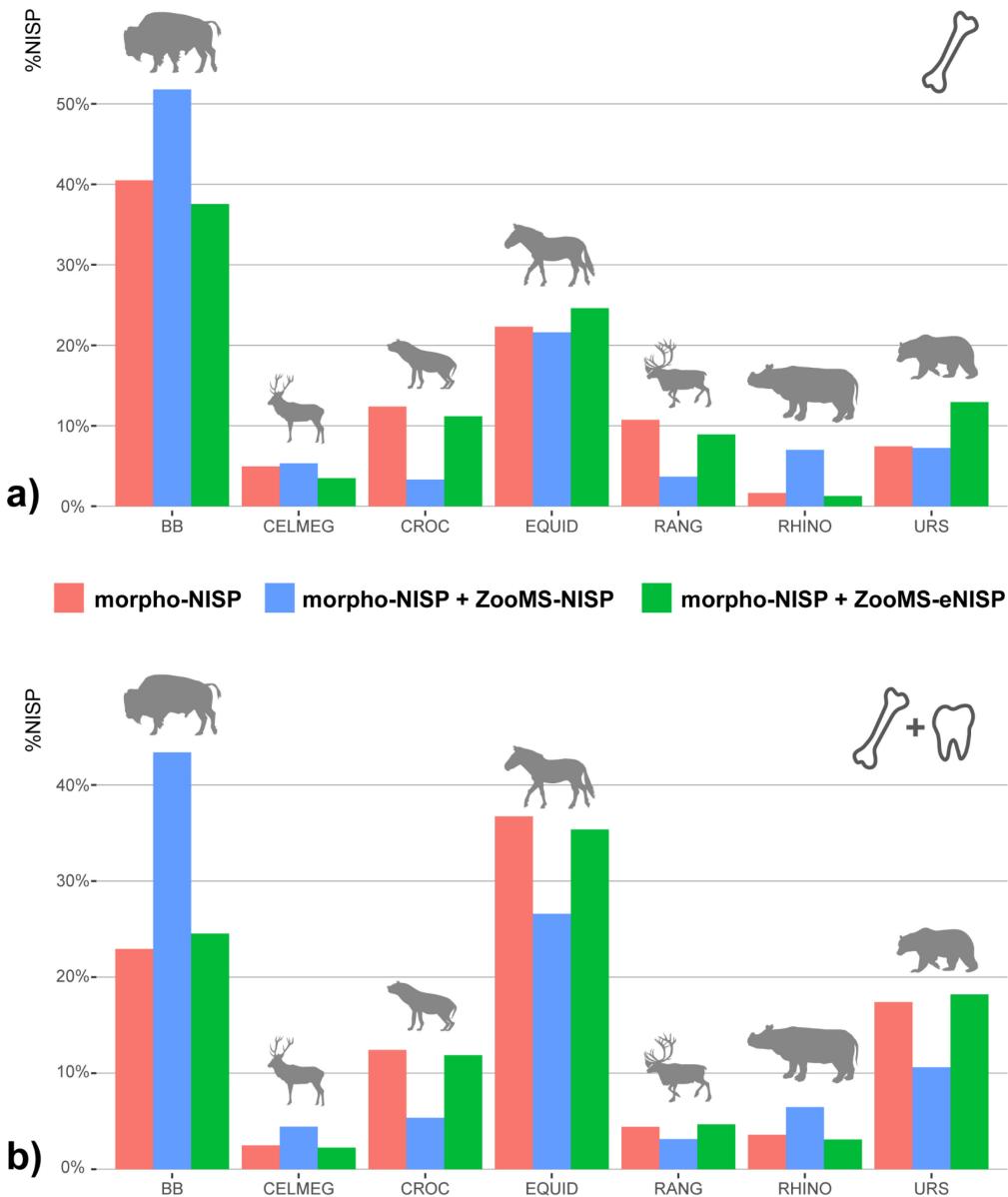


Figure 5. Species abundance (%NISP) derived from morpho-NISPs, ZooMS-NISPs, and ZooMS-eNISPs (a: only bones, b: bones and teeth). (See Table 2 caption for reference to the abbreviations used for each taxon.)

and the bone assemblage represents a complex mix of hyena- and human-accumulated bones. First and foremost, experimental work to better understand carcass fragmentation of different taxa would be very useful. Further work on the smaller fraction (<2cm, not considered at Cassenade) might also shed light on the differential (or identical?) fragmentation of bones per species.

Other questions are still left unanswered: how much bone granulometry is affected by site formation processes (such as water runoff), and can it drastically impact %NISP values? What drives the major differences in cranial/post-cranial proportions among taxa, and how can we integrate bone NISPs and tooth NISP? At Cassenade, horse teeth are relatively more abundant (see Figure 2), but patterns of tooth fragmentation could not be investigated here with

the ZooMS dataset (ZooMS were primarily carried out on bone fragments, leaving most tooth fragments aside). Are horse teeth fragmented in more pieces? Are smaller pieces of horse teeth easier to identify compared to other taxa, with their typical highly hypsodont morphology identifiable in small “splinters”? Were animal carcasses of different species processed differently? Explaining these patterns would require further work, and especially a further integration of ZooMS data with morphological datasets.

ARE BODY SIZE CLASSES REALLY USEFUL?

Many Paleolithic zooarchaeologists assign unidentifiable bone fragments to mammal size classes (e.g., for Eurasian taxa: Castel 1999; Costamagno 1999; Discamps 2011b; Fosse 1994; Morin 2004). Consecutively, NISP numbers by body

size classes can be integrated in zooarchaeological analyses to better discuss subsistence strategies, for example, prey choices, by comparing proportions of medium (e.g., reindeer) and larger (e.g., Bovinae and horse) ungulates (Rendu et al. 2019, 2023). Two lines of evidence question the need and interest of such approaches. First of all, several ZooMS studies highlighted inconsistencies between body size classes (as identified by zooarchaeologists) and proteomic identifications, with misidentifications leading to an underrepresentation of smaller ungulates (Ruebens et al. 2023, 2024 [this volume]; Sinet Mathiot et al. 2019, 2023). Secondly, the results obtained by the analysis of size and mass distributions at Cassenade show that bones of larger ungulates will break in a higher number of fragments, and thus these taxa will likely be overrepresented in NISPs numbers by body size classes. Weighing these fragments might counterbalance this issue (as in ZooMS-eNISP), yet, overall, the relevance of using body size classes for morphologically unidentified bones can be severely questioned. Remarkably, it is worth noting that the two biases affect species proportions in opposite ways—misidentifications might have a tendency to cause an under-representation of large ungulates, while fragmentation would induce their over-representation. In the absence of more detailed quantifications of each of these biases, it is impossible to predict how data on body size classes might be affected and thus, how zooarchaeologists could interpret them in terms of species proportions.

In the end, we question the interest of sorting long-bone shaft fragments by body size classes for at least three reasons: 1) ZooMS studies show that it is prone to risks of misidentification; 2) these risks of misidentification are increased by the fact that zooarchaeologists often rush this step during analysis of large collections, as it is a time-consuming process that provides little information; and, 3) NISP counts by body size classes are most probably highly biased by fragmentation issues. Body size classes are, however, probably more robust and useful when the skeletal element can be identified with precision.

CONCLUSION

ZooMS studies are already of huge interest to zooarchaeological research, and will likely be even more useful in the near future, for example, by identifying rare species (or at least species for which skeletons are rarely available in research institutions, such as *Megaloceros giganteus*, Rhinocerotidae, *Mammuthus primigenius*, or *Ovibos moschatus*), increasing faunal diversity at a given site (by increasing the total sample size of identifiable fragments), helping in the identification of elements for which taxonomic identification is difficult (e.g., bone industry, fetus bones) notably for specific skeletal elements (e.g., axial bones) or even portions of them (e.g., skull parts apart from maxillary and petrous portions). However, zooarchaeologists and proteomic specialists need to work closely together to find ways of fully integrating and exploiting their datasets to answer complex zooarchaeological questions (carcass transport, seasonality, taphonomic histories, etc.) rather than simply compare

their results and contrasting interpretations.

The Cassenade example calls for caution by demonstrating that: a) ZooMS-NISPs are affected by biases that render interpretations of species abundance derived from them unreliable, and, b) that the use of body size classes by zooarchaeologists is affected by the same biases and thus, at best, of little use. The biases introduced by bone fragmentation and differential identification might alter quantitative proportions of different species in a way that does not necessarily match the expectations of zooarchaeologists and proteomic specialists. Here, we propose the calculation of an adjusted ZooMS-eNISP, a site-specific measurement that seeks to estimate the number of extra fragments identified by ZooMS if all fragments were refitted to a size comparable to what is needed to be morphologically identifiable. The integration of ZooMS and morphological identifications is also rendered complex by the lower precision of ZooMS identifications for some taxa, as in the example of “Cervid/Saiga,” a problem that might be resolved in the future with further proteomic methodological research (e.g., SPIN, Ruether et al. 2022). Pending new research, lower precision ZooMS identifications are probably better integrated by grouping taxa together (e.g., as with the case of *Cervus elaphus* and *Megaloceros giganteus* in this study) or by testing several options and exploring their impact on interpretations (e.g., if all “Cervid” ZooMS identifications are attributed to one taxon or the other, what difference does it make in terms of interpretation of species abundance?). Further collaborative work between specialists should help in further integrating ZooMS data in zooarchaeological interpretations, and to obtain a more fine-grained reconstruction of species abundance and past human subsistence behavior.

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DATA AVAILABILITY STATEMENT

Supplementary data for this manuscript are available through Zenodo (<https://doi.org/10.5281/zenodo.12600092>).

This includes the entire Cassenade dataset used here (SI#1 and SI#2: morphological and ZooMS identifications, sizes, masses, etc.) and the R script used for making the figures and statistical tests (SI#3).

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