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Research Article

Dietary preferences and collagen to collagen prey-predator trophic discrimination factors ($\Delta^{13}C$, $\Delta^{15}N$) in late Pleistocene cave hyena

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Abstract

The spotted hyena (*Crocuta crocuta*) was an important large carnivore of Pleistocene ecosystems in Africa and Eurasia. Like its modern relatives, this obligate carnivore was adapted to crush and digest bones of its prey and absorb organic matter from bones more efficiently than any other carnivore. This difference in the nutrient resource use between hyenas and most other carnivores led to differences in the isotope flux and variation in the carbon and nitrogen isotopic composition. In our paper, we assess the prey-to-hyena collagen-to-collagen $\Delta^{13}C$ and $\Delta^{15}N$ trophic discrimination factor (TDF), a key parameter needed in mixing models used for quantitative reconstruction of diet. We analyzed a Pleistocene hyena den bone accumulation in Perspektywiczna Cave (Poland), with a preserved assemblage of remains containing both hyenas and a wide spectrum of their prey represented by digested bones. With the use of proteomics-based taxonomic identification (ZooMS), we estimated the proportion of prey species in the hyena diet. The modeled collagen-to-collagen TDFs are around +1.6‰ to +1.7‰ for $\delta^{13}C$ and around +3.4‰ to +3.5‰ for $\delta^{15}N$. This study provides new data on the dietary habits of this large carnivore and allows for a more accurate use of isotopic signals in modeling past hyena diets.

Keywords: paleontology, isotope ecology, paleoecology, diet reconstruction, methodology, trophic enrichment factor, spotted hyena, *Crocuta*, Perspektywiczna Cave, ZooMS

Introduction

The $\delta^{13}C$ and $\delta^{15}N$ values of animal tissues are used in paleoecology as proxies of trophic relationships (Hobson *et al.*, 2000; Ambrose and Katzenberg, 2002; Post, 2002; Hedges and Reynard, 2007; Koch, 2007; Bocherens, 2015). Ecological aspects such as diet, niche widths, niche overlaps, trophic positions, and food web structures can be tracked following the principles of isotope flux from the diet to the consumer's body (Bearhop *et al.*, 2004; Newsome *et al.*, 2007; Flaherty and Ben-David, 2010; Jabot *et al.*, 2017). This flux has been found by many studies to be regular and dependent on a diet's isotopic composition and the consumer's metabolism (Crowley *et al.*, 2010; Froehle *et al.*, 2010; Perga and Grey, 2010; Codron *et al.*, 2011, 2012, 2018; Mohan *et al.*, 2016; Stephens *et al.*, 2022, 2023).

Metabolic processes within an animal's body leads to isotopic discrimination in biochemical reactions and redistribution of isotopes between metabolites. A consequence is an isotopic shift, or fractionation, between an animal's body and its diet (Hedges and van Klinken, 2002; Bocherens and Drucker, 2003; Hahn *et al.*, 2012; Mohan *et al.*, 2016; Crowley *et al.*, 2020). This shift is a key parameter needed for isotope-based estimation of the use of dietary resources by animals, in particular in mixing models used for quantitative reconstruction of diet (Stephens *et al.*, 2022, 2023). This parameter is known as the trophic enrichment factor (TEF), trophic discrimination factor (TDF), or dietary isotopic fractionation function (DIFF) (Hedges and van Klinken, 2002; Caut *et al.*, 2009; Perga and Grey, 2010), and is usually denoted as Δ or ϵ. In this paper we call it TDF and denote it as Δ. The TDF can be established between the average diet or selected dietary components and the whole consumer body or its selected tissues or compounds. In paleoecology, one of the most popular TDFs is prey bone collagen to carnivore bone collagen TDF (Bocherens and Drucker 2003; Krajcarz *et al.*, 2018). The focus is on bone collagen because bones are usually among the few preserved tissues in an archaeological context.

The TDF of an animal species or individual is strongly related to its metabolism, diet, and lifestyle (Caut *et al.*, 2009; Perga and Grey, 2010; Poupin *et al.*, 2011; Codron *et al.*, 2018). Many aspects of natural feeding behavior (e.g., foraging, hunting, eating and digestion under stressful conditions, starvation), and thus natural metabolism, cannot be fully reproduced in experiments where no physical activity is needed to gain the food. Therefore in (paleo)-ecological applications the most appropriate TDFs are those established for free-living animals in their natural environments (Codron *et al.*, 2011; Stephens *et al.*, 2022, 2023). However, most of the published TDF values were obtained from feeding

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experiments in which animals are kept in cages and fed a specific, homogeneous diet. This is because sampling for TDF studies in a natural environment is difficult and ethically disputable. However, there are alternative approaches, including ecological monitoring methods (Newsome *et al*., 2010; Johnson *et al*., 2020, 2023) or sampling well-preserved paleontological taphocenoses or from stomach contents (Fox-Dobbs *et al*., 2007; Codron *et al*., 2012; Krajcarz *et al*., 2018, 2019). These approaches do not interfere with the natural behavior of the animals studied, are able to sample a wide range of their prey, and therefore allow for the calculation of a natural TDF.

Most carnivorous mammal-focused studies use the collagen-to-collagen TDF values of around +1.0‰ for $\Delta^{13}C$ and +3.4‰ for $\Delta^{15}N$ (Hedges and Reynard, 2007; Bocherens, 2015; Bocherens *et al*., 2015; Wißing *et al*., 2016, 2019; Drucker, 2022), which are average values established through experiments and wild population studies (Minagawa and Wada 1984; Schoeninger and DeNiro, 1984; Schoeninger, 1985; Ambrose and DeNiro, 1986; Schwarcz, 1991; Szepanski *et al*., 1999; Bocherens and Drucker, 2003; Fox-Dobbs *et al*., 2007). However, an in-depth analysis of the published TDF databases revealed that TDF is taxonomically specific (Caut *et al*., 2009; Bond and Diamond, 2011; Stephens *et al*., 2022, 2023). Moreover, TDF estimates are subject to uncertainty because of tissue-specific isotope turnover and factors that can affect TDF, such as diet quality, body size, or nitrogen excretion pathway used by the consumer (Minagawa and Wada, 1984; Ben-David *et al*., 2001; Vanderklift and Ponsard, 2003; Mohan *et al*., 2016; Whiteman *et al*., 2021). Thus, the average or randomly selected TDF values can be non-representative for a studied species, and it is recommended to select the TDF that is known for the same or closely related species (Stephens *et al*., 2022, 2023). Nevertheless, even selecting the TDF values of a close relative may be problematic given that $\Delta^{13}C$ and $\Delta^{15}N$ can vary by as much as several per mils within phylogenetic groups (see review in Stephens *et al*., 2023). It is therefore necessary to establish collagen-to-collagen TDF values independently for each important consumer species subjected to paleoecological study.

The spotted hyena, *Crocuta crocuta*, was an important faunal component of Pleistocene steppe and savanna ecosystems in Africa, Europe, and Asia (Kurtén, 1968; Kahlke, 1999). Pleistocene populations, often referred to as cave hyenas, were previously grouped into a separate subspecies or species, *C. c. spelaea* or *C. spelaea*, but currently are considered the same species (Westbury *et al*., 2020; Krajcarz *et al*., 2023); however, their heterospecific taxonomy is still postulated (Lewis and Werdelin, 2022). Having an abundant fossil record and posing an important threat and competition to early humans, hyenas are of particular interest in Quaternary studies, especially in archaeology, anthropology, paleogeography and paleoecology, including isotopic paleoecology (Stiner, 1994, 2004; Brugal *et al*., 1997; Fosse, 1999; Enloe *et al*., 2000; Johnston, 2001; Marra *et al*., 2004; Villa *et al*., 2004; Diedrich and Žák, 2006; Dusseldorp, 2011, 2013; Stock and Semmens, 2016; Gatta *et al*., 2019; Jimenez *et al*., 2019; Krajcarz *et al*., 2023). However, the spotted hyena has unique dietary habits and digestive physiology that may affect its TDF. Hyenas are obligate carnivores, adapted to crush and digest bones of their prey (Kruuk, 1972). Their highly efficient digestive system allows them to absorb organic matter from bones more efficiently than any other carnivore (Kruuk, 1972). A genetic study by Westbury *et al*. (2021) confirmed that the hyena's specific feeding behavior—scavenging on potentially infectious carrion and consumption of large quantities of bones—was facilitated by genetic adaptations to the immune and digestive systems. The ability to consume much larger quantities of bone collagen than any other carnivore allows hyenas to effectively exploit a nutrient source that is not accessible for other predators, even if feeding on the same prey. This discrepancy in the resource use between hyenas and most of other carnivores leads to differences in the isotope flux and, as a consequence, possible variation in the collagen-to-collagen TDF.

The bone collecting behavior of spotted hyenas makes them good candidates for field-derived estimates of TDF case studies in natural ecosystems. In this paper, we assess the prey–predator collagen-to-collagen TDF ($\Delta^{13}C$ and $\Delta^{15}N$) for the Pleistocene cave hyena through isotopic analysis of the fossil bone accumulation in Perspektywiczna Cave (Poland) (Krajcarz *et al*., 2023). Utilizing zooarchaeology by mass spectrometry (ZooMS), we determined the prey profile that makes up the hyena diet based on digested bone fragments (i.e., bones that were not just collected, but definitely eaten by hyenas). Finally, we compared the isotopic TDF obtained for hyena with published TDFs for other carnivores to assess its variability. This study presents new data on the dietary habits of this famous large carnivore and will allow for a more accurate use of isotopic signals in modeling past hyena diets.

Material and Methods

Perspektywiczna Cave hyena den

Perspektywiczna Cave is located in Udorka Valley (50°26′33.5″N, 19°46′1.5″E), in the middle part of Kraków-Częstochowa Upland, southern Poland. The radiocarbon, stable isotope, and paleogenetic study (Krajcarz *et al*., 2023) revealed that during the late Pleistocene the cave served as a den occupied by cave hyena clans for around 20,000 years, during the MIS 3 stage (ca. 55–34 ka BP). The hyena den taphocenosis is composed of hyena remains, both adults and juveniles, and bones of hyena prey, including chewed and digested bone fragments. The taphocenosis is preserved in situ in the upper chamber of the cave and within colluvial lobes that were re-deposited to the lower chamber. Combined stable isotope analysis and radiocarbon dating revealed that, beside the long period of occupation, the isotopic ecology of the Perspektywiczna Cave hyenas remained unchanged and their carbon–nitrogen isospace niche was relatively narrow (Krajcarz *et al*., 2023). Therefore, the entire taphocenosis represents a similar and stable ecology, despite its long chronology.

Material

Our main research specimens are bones of spotted hyenas and their prey from Perspektywiczna Cave. Most of the bones excavated from hyena den-related strata are likely related to hyena diet. However, it is possible that some part of the assemblage may represent other depositional agents, in particular the activity of other carnivores (e.g., cave lion [*Panthera spelaea*], wolf [*Canis lupus*], red fox [*Vulpes vulpes*], and polar fox [*Vulpes lagopus*]), or cave bear (*Ursus spelaeus*) hibernation (Krajcarz *et al*., 2016). In an attempt to sample only those bones that were related to hyena diet, we selected bone fragments with clear evidence of heavy digestion, thus most likely coming from prey consumed by hyenas. Sample selection criteria were: (1) bone surface destruction typical for digestion by hyena (i.e., rounded,

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smoothed morphology, corrosive perforation; Fernández-Jalvo and Andrews, 2016); (2) size allowing sampling for ZooMS, stable isotopes, and retaining some leftover for archiving (> 5 g); and (3) certain stratigraphic position (i.e., hyena den-related layer 7c). In total, 108 fragments were selected, all of which were sampled for ZooMS taxonomic analysis (details of research material are available in the PANGAEA repository: https://doi.org/10.1594/PANGAEA.971340).

Most of these fragments were then sampled for stable isotope analysis, except for reindeer (*Rangifer tarandus*), which was the most abundant species identified among the studied bones. In the case of reindeer, we analyzed only 9 specimens of the 47 total identified. The variability of obtained reindeer isotopic values was rather low (Figure 1) and the values were similar to those obtained previously (Krajcarz *et al*., 2016); therefore, we assumed that the sampled portion is an accurate representation of the reindeer present at the site.

Some of the Perspektywiczna Cave species were previously analyzed for carbon and nitrogen stable isotopes and these results have been published already (Krajcarz *et al*., 2016, 2023). These data were based mostly on non-digested, morphologically identified bone remains. Although the direct affiliation of these bones with the hyena den was in some cases uncertain, they represented the same taxa as analyzed within the digested assemblage. Therefore, we included isotopic values of these bones in our database and used them together with those for digested bones to establish the average isotopic signal of identified taxa.

ZooMS analysis

ZooMS proteomics analysis was performed at BioArCh (Biology, Archaeology, and Chemistry departments), University of York. Collagen was extracted using the acid insoluble protocol commonly used for ZooMS (Welker *et al*., 2015). In brief, a subsample of bone was demineralized in 250 µL of 0.6 M HCl (4°C for 48 hours), washed once with 200 µL 0.1 M sodium hydroxide (NaOH) to remove possible humic contamination, and washed twice with 200 µL 50 mM ammonium bicarbonate (AmBic). After washing, 200 µL of 50 mM AmBic was added to the sample, which was incubated at 65°C for 1 hour to gelatinize any available collagen into solution. Following gelatinization, 100 µL of the supernatant was transferred to a new microfuge tube while the remaining sample and 100 µL AmBic was stored at −20°C for further analysis, if required.

The collagen extract was digested overnight (ca. 18 hours) with the enzyme trypsin at 37°C; digestion was stopped with the addition of 5% v/v trifluoroacetic acid (TFA). The resulting peptides were purified using C_{18} ZipTip pipette tips and eluted in 100 µL of conditioning solution (0.1% TFA in 50:50 acetonitrile:water [ACN:water]). One µL of sample was spotted on to a Bruker ground steel target plate and mixed with 1 µL of matrix (alpha-cyano-4-hydroxycinnamic acid [CHCA]). Each sample was spotted in triplicate alongside calibration standards, and the plate was run on a Bruker Ultraflex III MALDI-ToF (matrix assisted laser desorption ionization time of flight) mass spectrometer. The laser intensity was set at 40–55% and a mass range of 800–4000 Daltons (Da). Peptide masses below 650 Da were suppressed. Each sample was externally calibrated against an adjacent spot containing a mixture of six peptides (des-Arg1 Bradykinn m/z = 904.681, Angiotensin I m/z = 1295.685, Glu1-Fibrino- peptide B m/z = 1750.677, ACTH [1–17 clip] m/z = 2093.086, ACTH [18–39 clip] m/z = 2465.198 and ACTH [7–38 clip] m/z = 3657.929).

The spectra were analyzed using mMass, an open-source mass spectrometry tool (Strohalm *et al*., 2010). The three replicates were averaged, and the resulting averaged spectrum was cropped to 800–3500 m/z and peak picked using a minimum signal/noise of 4. The peak list was compared to a list of published markers to provide possible identifications (Buckley *et al*., 2009; Buckley and Collins, 2011; Kirby *et al*., 2013; Welker *et al*., 2016; Culley *et al*., 2021).

Stable isotope analysis

Approximately 0.5-g samples (min. 0.1 g to max 0.5 g) were cut off using a rotary diamond-coated saw and crushed to grain size less than 0.7 mm. The extraction followed Bocherens *et al*. (1997). The detailed protocol is available at: dx.doi.org/10.17504/protocols.io.8epv5rrp4g1b/v1. In brief, the extraction protocol included following steps: (1) 1M HCl treatment (room temp., 20 min); (2) filtration and residuum collection; (3) 0.125M NaOH treatment (room temp., 20 h); (4) filtration and residuum collection; (5) gelatinization in pH = 2 HCl (temp. 100°C, 17 h); (6) filtration and supernatant collection; and (7) lyophilization. Internal standards of modern elk and seal bones underwent collagen extraction and measurements together with fossil samples for quality control over the process. The quality of extracted collagen was checked by measuring the C and N content and calculating the C:N atomic ratio. We accepted greater than 5% for the N content and 2.9–3.6 for C:N atomic ratio (DeNiro, 1985; Ambrose, 1990).

Elemental analysis and isotopic measurements were performed at facilities of the Geoecology Working Group of the University of

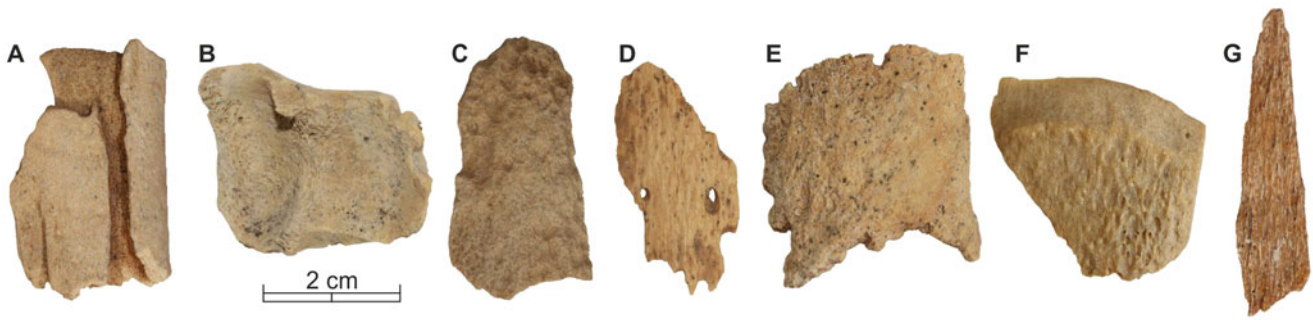

Figure 1. Exemplary digested bone fragments from Perspektywiczna Cave: (**A**) reindeer antler (HPe 64, ZooMSPC015); (**B**) reindeer tibia epiphysis (ZooMSPC035); (**C**) large cervid antler, identified by ZooMS as bovid/cervid (HPe 20, ZooMSPC018); (**D**) rhinoceros (HPe 52, ZooMSPC009); (**E**) rhinoceros (HPe 57, ZooMSPC066); (**F**) mammoth (HPe 40, ZooMSPC064); (**G**) mammoth (HPe 41 and ZooMSPC069).

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Tübingen (Germany) using a CHNOS Vario Isotope Cube elemental analyzer (Elementar) in conjunction with an IsoPrime visION isotope ratio mass spectrometer (Elementar). The international standards (USGS-40 and USGS-41a) and two in-house reference materials (modern collagen of camel and elk) were used to calibrate the results. An analytical error (1σ) below 0.1‰ and 0.2‰ was determined for $\delta^{13}C$ and $\delta^{15}N$, respectively. Selected measurements ($n = 9$, see data in repository https://doi.org/10.1594/PANGAEA.971340) were repeated in separate batches, according to the recommendations of Szpak *et al.* (2017).

A subset of collagen samples ($n = 11$, see Supplementary Information: sheet 2 for details) was randomly selected for cross-laboratory checking in the Institute of Geological Sciences of the Polish Academy of Sciences (Warsaw, Poland). Isotopic measurements were performed using a Flash EA 1112HT elemental analyzer (Thermo Scientific) in conjunction with a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific). International standards (USGS-40, USGS-41, and IAEA-600) were used for 3-point calibration. Analytical errors (1σ) recorded over a long time of measurements were below 0.33‰ and 0.43‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Isotopic values were expressed as δ (delta; an isotopic ratio over the ratio of an appropriate reference) in parts per mil (‰), according to the formula:

$$\delta^{x}E = \frac{R(^{x}E/^{y}E)_{sample} - R(^{x}E/^{y}E)_{reference}}{R(^{x}E/^{y}E)_{reference}} \cdot 1000$$

where ^{x}E is ^{13}C or ^{15}N, and $R(^{x}E/^{y}E)$ is a $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio (Coplen, 2011). The references were VPDB (Vienna Pee Dee belemnite) for carbon, and atmospheric nitrogen (AIR N_2) for nitrogen.

Mean diet

To calculate the isotopic composition of a mean diet, we followed the methodology of Krajcarz *et al.* (2018). We used each prey taxon's mean isotopic value (arithmetic mean of all isotopic data available for a given taxon in the Perspektywiczna Cave dataset, separately for $\delta^{13}C$ and $\delta^{15}N$) and a proportion of each taxon in the hyena's diet, called here P_i. For calculation of mean isotopic value of a taxon, we used isotopic data available for both morphologically and ZooMS-identified bone specimens; for estimation of the P_i, we used all digested bone remains, including morphologically and ZooMS-identified bones with digestion marks (see reposited data in https://doi.org/10.1594/PANGAEA.971340). Since our data were limited to the number of identified specimens (NISP) for each taxon, that had to be transformed into consumed biomass. This is crucial because, in the case of highly fragmented and anatomically unidentified bone fragments, the size and type of a bone cannot be established and thus similar numbers of bone fragments coming from different taxa do not necessarily reflect a comparable amount of consumed nutrients. We adopted the methodology of Ackerman *et al.* (1984) to transform the NISP of each taxon's bone fragments into that taxon's consumed biomass, following the formula:

$$ABC_i = (1.98 + 0.035Bm_i)NISP_i$$

where ABC_i is the Ackerman *et al.* (1984) approximation of biomass consumed, and Bm_i is an i^{th} taxon's average body mass in kg (after Brook and Bowman, 2004), and i is a given taxon in the diet. Differences in body mass between juveniles and adults would affect the calculation. However, because the individual ages of most our specimens are unknown, we assumed the adult body mass.

The weakness of the *ABC* formula, initially developed for analysis of scats, is the assumption that each bone fragment represents a separate prey individual. Being aware that this may not always be the case, we nevertheless assumed that each bone fragment, excavated from a hyena den that was active for 20,000 years, likely represents another individual and possibly another accumulation interval. Another weakness is that Ackerman *et al.*'s (1984) formula was experimentally established for prey in the range of 2–70 kg of body mass, while our dataset includes animals of a much larger body mass (e.g., bison [*Bison priscus*], rhinoceros [*Coelodonta antiquitatis*], mammoth [*Mammuthus primigenius*]). Being aware that the regression may be non-linear and thus biased in the case of large prey, we also calculated the biomass consumed using a simple approach of:

$$BC_i = Bm_i \cdot NISP_i$$

where BC_i is i^{th} taxon's biomass consumed based directly on the product of the i^{th} taxon's NISP and body mass. The relative percent of biomass consumed calculated following the *ABC* and the *BC* formulas provided almost identical values (see Supplementary Information: sheet 1). The P_i was then calculated as each taxon's participation in the total biomass consumed, as:

$$P_i = \frac{\mathrm{ABC}_i}{\sum_{i=1}^{n} ABC_i}$$

Then, the mean isotopic value of the hyena diet was calculated as a weight balance of all prey taxa's mean isotopic values (either $\delta^{13}C$ or $\delta^{15}N$), according to the formula:

$$\delta^{x}E_{diet} = \frac{\sum_{i=1}^{n} P_i \delta^{x}E_i}{\sum_{i=1}^{n} P_i}$$

where $\delta^{x}E_{diet}$ is the mean isotopic value of hyena diet (either $\delta^{13}C$ or $\delta^{15}N$) and $\delta^{x}E_i$ is the mean (arithmetic) isotopic value of an i^{th} taxon. The formula is similar to the one used by Greer *et al.* (2015) but simplified through assumption that all dietary components have similar chemical composition (all are mammalian prey), so each component contributed a similar proportion of C-bearing and N-bearing compounds (but see our Discussion section for a discussion of this assumption).

Gnawed and digested hyena bones were present in the assemblage, thus indicating that hyena remains could constitute some portion of nutrients ingested by hyenas; this could have been sporadic, but the importance of this nutrient source is hard to establish. Therefore, further calculations were performed independently for two assumptions: of hyenas themselves being and not being included in the diet, resulting in two independent mean diet models.

The standard error of the mean diet's isotopic composition was calculated as propagation error, according to the formula:

$$\sigma_{diet} = \frac{1}{\sum_{i=1}^{n} P_i} \sqrt{\sum_{i=1}^{n} (p_i \sigma_i)^2}$$

where σ_{diet} is the standard error for a mean diet and σ_i is standard deviation of an i^{th} taxon's mean isotopic value (either $\delta^{13}C$ or

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δ^{15}N). All calculations were performed using MS Excel software; spreadsheets are available in the Supplementary Information: sheet 1.

TDF calculation

Two independent TDFs were calculated for two assumptions of hyena bones either being included or excluded in the diet, as differences between hyena's mean isotopic value and modeled hyena's mean diet isotopic values, according to formula:

$$\Delta^{x}E_{hyena} = \delta^{x}E_{hyena} - \delta^{x}E_{diet}$$

The standard error of TDF was calculated as a propagation error for the difference of mean hyena isotopic value and mean diet isotopic value (either δ^{13}C or δ^{15}N), according to the formula:

$$\sigma_{TEF} = \sqrt{\sigma^{2}_{hyena} + \sigma^{2}_{diet}}$$

where σ_{TEF} is standard error for TDF and σ_{hyena} is standard deviation of a hyena's mean isotopic value (either δ^{13}C or δ^{15}N). All calculations were performed in the MS Excel software; spreadsheets are available in the Supplementary Information: sheet 1.

Theoretical TDF calculation in SIDER

The R package SIDER (Healy *et al.*, 2018) allows for prediction of species-specific $TDF_{tissue\text{-}diet}$ based on Bayesian inference and phylogenetic regression models of a large built-in dataset composed of lab-derived TDFs (Healy *et al.*, 2018; Stephens *et al.*, 2022). We used this method to compare our results obtained for the paleontological assemblage with theoretical prediction. For the calculation with SIDER, we used the software R (version 4.3.2) in RStudio (version 2023.12.1 Build 402). We updated the TDF database with data from Stephens *et al.* (2022) and calculated the $\Delta^{13}C_{diet\text{-}collagen}$ and $\Delta^{15}N_{diet\text{-}collagen}$ for spotted hyena with the following recipeSider settings: species = "Crocuta_crocuta", habitat = "terrestrial", taxonomic.class = "mammalia", tissue = "collagen", diet.type = "carnivore". Our R script with further technical details is provided in the PANDORA repository (CEMP SITE Community, https://www.doi.org/10.48493/ak8a-z502).

In contrast to the collagen-to-collagen TDF (referred to as $TDF_{collagen\text{-}collagen}$) obtained in our study based on fossil bones, the SIDER output is collagen-to-diet TDF ($TDF_{collagen\text{-}diet}$), which represents the isotopic offset between the predator's bone collagen and its entire (averaged) diet. In order to make both TDF models comparable, we converted our $TDF_{collagen\text{-}collagen}$ to TDFs related to other dietary tissues. Since hyenas eat not only meat but also bones, hooves, and hair, we converted the $TDF_{collagen\text{-}collagen}$ to $TDF_{collagen\text{-}meat}$ and $TDF_{collagen\text{-}hair}$ (the first "$_{collagen}$" in the subscript refers to the consumer tissue [i.e., the hyena bone collagen], and the second word [i.e., "$_{collagen}$", "$_{meat}$", or "$_{hair}$"], refers to the respective prey tissue). These tissue-specific TDFs represent a breakdown of the $TDF_{collagen\text{-}diet}$, which is expected to be within their range. To calculate these TDFs, we used the mean isotopic differences between bone collagen and muscle (2.60 ± 0.02‰ for δ^{13}C and -0.12 ± 0.02‰ for δ^{15}N), and between bone collagen and keratin (1.27 ± 0.03‰ for δ^{13}C and -0.18 ± 0.02‰ for δ^{15}N), which are the averages of respective differences published by Stephens *et al.* (2023).

Results

ZooMS results

Taxa identified in the studied assemblage using ZooMS are shown in Figure 2 and can be found in the repositories (https://doi.org/10.1594/PANGAEA.971340 and https://doi.org/10.1594/PANGAEA.971344). ZooMS identification was successful in all of the 108 sampled specimens, of which 90 were identified as a specific ZooMS taxon (Bear, Caribou/Reindeer, Cattle, Elephant/Mammoth, Horse, Rabbit/Hare, and Rhino), and 18 were classified into a group of closely related families (Bovidae/Cervidae, Carnivora: Canid [Fox/Dog/Wolf/Coyote/Dhole], and Carnivora: Lion/Tiger/Hyena). Specimens identified as Carnivora: Lion/Tiger/Hyena were further tested with mtDNA analysis and confirmed to belong to spotted hyena (Krajcarz *et al.*, 2023). Because the studied assemblage refers to Central European late Pleistocene mammals, we assumed that the ZooMS taxa likely corresponded to the following species: bear: cave bear or brown bear (most likely *Ursus ingressus*, identified in several specimens by mtDNA analysis (Gretzinger *et al.*, 2019); caribou/reindeer: reindeer (*Rangifer tarandus*); cattle: bison/aurochs (*Bison*/*Bos*); elephant/mammoth: mammoth (*Mammuthus primigenius*); horse: wild horse (*Equus* sp.); rabbit/hare: leporid (most likely *Lepus* sp.); and rhino: woolly rhinoceros (*Coelodonta antiquitatis*). The group of Bovidae/Cervidae most probably represents giant deer (*Megaloceros giganteus*), red deer (*Cervus elaphus*), or saiga (*Saiga tatarica*), and is referred to in the following text as bovid/cervid.

Most of the ZooMS-recognized taxa were also identified morphologically in the osteological material. The only exception is mammoth, which was not found among the morphologically identifiable bones. Reindeer is the most common species in the analyzed assemblage of digested bone fragments (44% of number of identified specimens [NISP]). The next most represented taxa of herbivores are woolly rhinoceros (11%), mammoth (9%), horse (6%), and bison/aurochs (5%). Bovids/cervids (8%) are also relatively abundant. Digested bone fragments of hyena constitute 7% of the NISP, the same proportion that was recorded for bear. Leporids (2%) and canids (1%) constituted only a small amount of the assemblage (Figure 2).

Stable isotope results

The C:N atomic ratios in 138 out of 141 collagen extracts were in the range 3.2–3.5 (details in the repository https://doi.org/10.1594/PANGAEA.971340), which is within the acceptable range for uncontaminated, undegraded collagen (DeNiro, 1985). Three samples (HPe 15, HPe 29, HPe 78) had high C:N atomic ratios, beyond the acceptance range, and were discarded from further analysis. The cross-lab checked δ^{13}C and δ^{15}N values of selected samples showed very good inter-lab reproducibility: the average difference for δ^{13}C was 0.4‰ with regression's $R^2 > 0.98$, and for δ^{15}N the average difference was 0.1‰ with $R^2 > 0.99$ (see Supplementary Information: sheet 2 for details).

Bone collagen of hyenas exhibited the highest δ^{13}C and δ^{15}N values among studied taxa (Figure 3; Table 1; data in the repository https://doi.org/10.1594/PANGAEA.971340). Average isotopic values of ungulates stay in the range: δ^{13}C from −19.3‰ to −21.7‰, and δ^{15}N from 2.5‰ to 6.8‰. The most extreme values in this group were exhibited by reindeer (the highest δ^{13}C and the lowest δ^{15}N values) and mammoth (the lowest δ^{13}C and the highest δ^{15}N values). Specimens identified as bears have relatively low δ^{13}C

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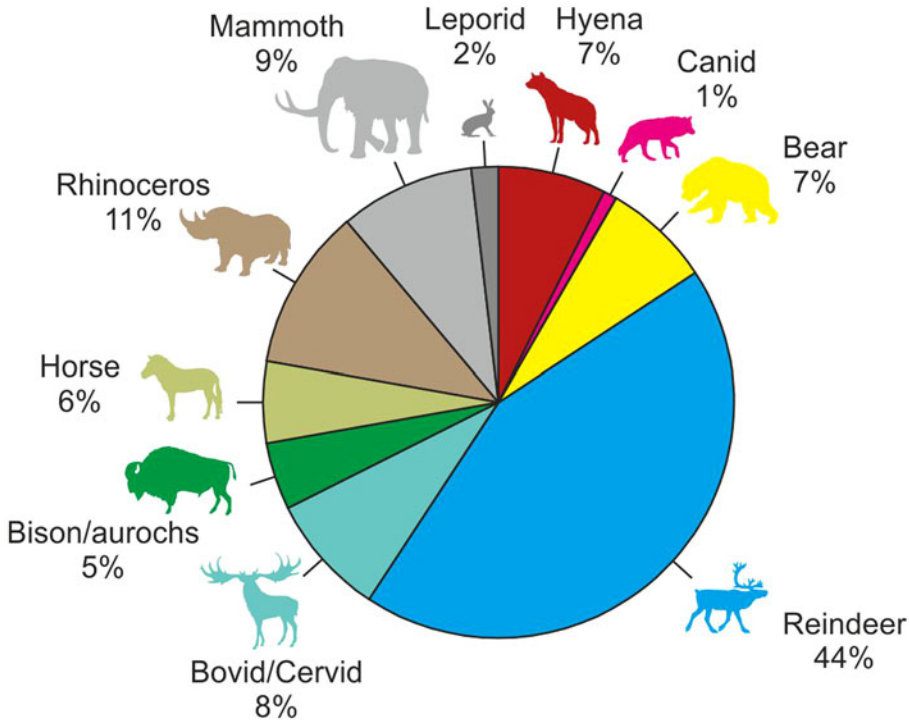

Figure 2. Taxonomic composition of the studied digested bone fragments from the Perspektywiczna Cave hyena den according to ZooMS identification (see text for explanation of taxonomic attribution).

and low δ^{15}N values, placing them in the range expected for Pleistocene cave bears (Krajcarz *et al.*, 2016). Few specimens of the bovid/cervid group are isotopically similar to reindeer, while most of them are within the isospace of bison/aurochs and rhinoceros.

Mean diet and TDF

Calculations of hyenas' mean diet isotopic signal according to Ackerman's index of consumed biomass and the straightforward

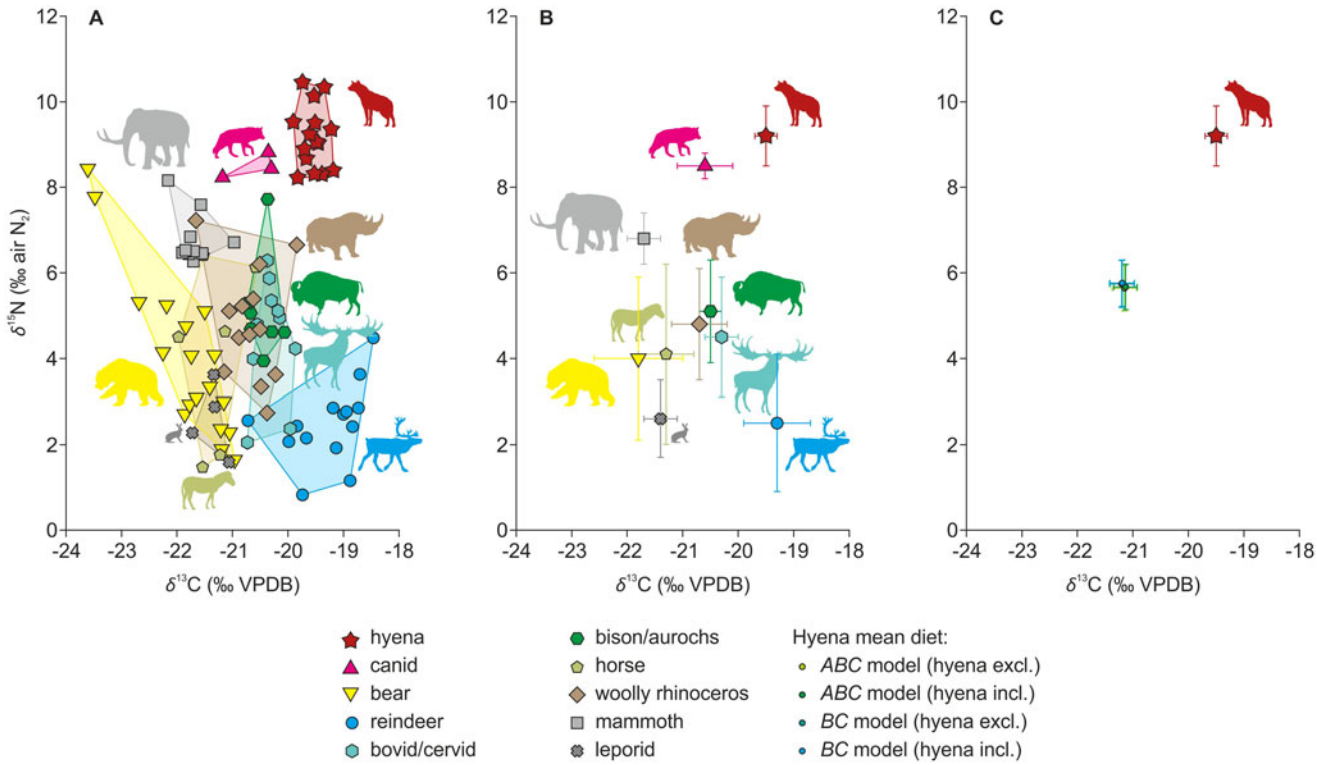

Figure 3. Stable isotope data for the Perspektywiczna Cave hyena den taphocenosis: (**A**) all specimens and convex hulls for taxa; (**B**) average value and standard deviation (1σ) for each taxon; (**C**) hyena and its modeled mean diet average values with standard errors (1σ). BC = biomass consumed; ABC = biomass consumed using methodology of Ackerman *et al.* (1984) to transform the NISP of each taxon's bone fragments into that taxon's consumed biomass; VPDB = Vienna Pee Dee belemnite.

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Table 1. Stable isotope results; SD = standard deviation; NISP = number of specimens identified by ZooMS; N Iso-ZooMS = number of ZooMS-identified specimens used for stable isotope analysis; N Iso-Morph = number of morphologically identified specimens additionally used for stable isotope analysis (mostly published data, see https://doi.org/10.1594/PANGAEA.971340)

Taxon	NISP	N Iso-ZooMS	N Iso-Morph	δ^{13}C mean	δ^{13}C SD	δ^{15}N mean	δ^{15}N SD
Hyena	8	7	8	−19.5	0.2	9.2	0.7
Canid	1	1	2	−20.6	0.5	8.5	0.3
Bear	8	7	10	−21.8	0.8	4.0	1.9
Reindeer	47	9	5	−19.3	0.6	2.5	1.6
Bovid/Cervid	9	9	1	−20.3	0.3	4.5	1.4
Bison/aurochs	5	5	2	−20.5	0.2	5.1	1.2
Horse	6	6	0	−21.3	0.5	4.1	2.1
Woolly rhinoceros	12	12	1	−20.7	0.5	4.8	1.3
Mammoth	10	10	0	−21.7	0.3	6.8	0.6
Leporid	2	2	2	−21.4	0.3	2.6	0.9

Table 2. Isotopic composition of the Perspektywiczna Cave hyena's mean diet (σ = standard error)

	mean diet δ^{13}C		mean diet δ^{15}N	
Mean diet model	avg	σ	avg	σ
ABC (Ackerman's index of biomass consumed)				
hyena excluded from diet	−21.15	0.22	5.65	0.53
hyena included into diet	−21.14	0.21	5.68	0.53
BC (simple biomass consumed estimation)				
hyena excluded from diet	−21.20	0.22	5.75	0.55
hyena included into diet	−21.19	0.22	5.76	0.55

consumed biomass estimations gave similar results (Table 2). The modeled collagen-to-collagen diet-to-hyena TDFs are around +1.6‰ to +1.7‰ for δ^{13}C and around +3.4‰ to +3.5‰ for δ^{15}N (Table 3). The SIDER model provides the theoretical $TDF_{collagen\text{-}diet}$ that is in the range of collagen-to-variable dietary tissues TDFs for δ^{15}N, but lower than collagen-to-variable tissues TDFs for δ^{13}C (Table 4). However, the SIDER's $TDF_{collagen\text{-}diet}$ has a large standard error.

Table 3. Trophic discrimination factor (TDF) between the Perspektywiczna Cave hyena's mean diet bone collagen and the hyena's bone collagen (σ stands for standard error)

	Δ^{13}C		Δ^{15}N	
Mean diet model	TDF	σ	TDF	σ
ABC (Ackerman's index of biomass consumed)				
hyena excluded from diet	1.61	0.30	3.52	0.91
hyena included into diet	1.60	0.30	3.49	0.90
BC (simple biomass consumed estimation)				
hyena excluded from diet	1.66	0.30	3.42	0.92
hyena included into diet	1.65	0.30	3.40	0.91

Discussion

Perspektywiczna Cave hyenas' prey choices

Modern spotted hyenas are often considered scavengers, but in fact they exploit a variety of foraging behaviors, including scavenging, as well as solitary and group hunting (Kruuk, 1972; Bohm and Höner, 2015). They are opportunists, and their prey selection reflects the relative abundance of the available herbivores in the environment (Kruuk, 1972; Bunn, 1983; Höner *et al*., 2005; Trinkel, 2010). Hyenas effectively prey on ungulates of body mass ranging from 20 to 300 kg (Kruuk, 1972; Cooper *et al*., 1999; Höner *et al*., 2005; Hayward, 2006). Larger species are usually available to hyenas as carrion or are occasionally hunted when either young or injured (Cooper *et al*., 1999; Holekamp and Dloniak, 2010; Bohm and Höner, 2015). Studies of modern

Table 4. Collagen-to-diet TDF (trophic discrimination factor) models (σ = error)

	Δ^{13}C		Δ^{15}N	
TDF models	TDF	σ	TDF	σ
TDF excluding hyenas from diet (*ABC*)				
collagen-to-collagen	1.61	0.30	3.52	0.91
collagen-to-meat	4.21	0.32	3.40	0.93
collagen-to-hair	2.88	0.33	3.34	0.93
TDF including hyenas in diet (*ABC*)				
collagen-to-collagen	1.60	0.30	3.49	0.90
collagen-to-meat	4.20	0.32	3.37	0.92
collagen-to-hair	2.87	0.33	3.31	0.92
TDF of the SIDER model				
collagen-to-diet	4.34	1.94	2.86	1.29

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African populations show that the spotted hyenas consume primarily species of around 40–400 kg body mass (Kruuk, 1972; Cooper *et al*., 1999; Trinkel, 2010; Fester *et al*., 2021). Small prey, such as porcupines, hares, or jackals, were reported among hyena kills, but in a very low amount, less than 1% (Kruuk, 1972; Cooper *et al*., 1999).

Spotted hyenas are bone collectors. They tend to transport parts of their prey into dens, where the remains accumulate over time (Sutcliffe, 1970; Skinner *et al*., 1986; Pokines and Kerbis Peterhans, 2007; Ruff *et al*., 2010). Gnawed, digested and regurgitated bone fragments also accumulate in dens (Kruuk, 1972; Hill, 1989).

Pleistocene cave hyenas are genetically very close to modern African spotted hyenas (Rohland *et al*., 2018; Westbury *et al*., 2020; Krajcarz *et al*., 2023) and fossil bone accumulations created by them share characteristics of their extant relatives (Brugal *et al*., 1997; Diedrich and Žák, 2006; Diedrich, 2010; Mangano, 2011; Jimenez *et al*., 2019; Rivals *et al*., 2022). Among the most abundant prey species usually identified in European hyena dens are woolly rhinoceros, horse, and cave bear; rarely mammoth, reindeer and other cervids, bison, and others (Diedrich and Žák, 2006; Diedrich, 2010, 2012; Dusseldorp, 2011; Fourvel *et al*., 2012; Discamps, 2014).

In the Perspektywiczna Cave, the prey spectrum of the hyena den taphocenosis shows flexible prey choice. The range of prey taxa is similar to that known from other sites and represents body masses ranging from around 60 kg (reindeer) to several tons (woolly rhinoceros and mammoth; see Supplementary Information: sheet 1 for body mass). The most prominent characteristics of the Perspektywiczna Cave assemblage is the high number of reindeer and mammoth. High reindeer percentage was identified at some hyena den sites in Central Europe, but never exceeding 15% of the total NISP (Diedrich and Žák, 2006; Diedrich, 2010), while the presence of mammoth remains was rarely noticed or disputable (Diedrich, 2010; Germonpré *et al*., 2014; Jimenez *et al*., 2019). The study by Sinet-Mathiot *et al*. (2019) revealed significant discrepancies between morphologically and molecularly identified taxa representation. Our ZooMS results also reveal that the taphonomic processes determining preservation of bones may cause a discrepancy between what was actually accumulated and what was well preserved. Smaller prey items, such as reindeer, are easier to be transported to the den and completely consumed, where their remains may accumulate in larger numbers but could be heavily damaged (Dusseldorp, 2011, 2013). In contrast, only few skeletal elements of large-body-mass animals would be transported from the kill or feeding site to a den, but their large size allows for better preservation. The presence of the smaller species may therefore be underestimated in hyena den assemblages when identified by bone morphology alone.

Pitfalls of the mean diet estimation

While the Perspektywiczna Cave fossil assemblage reflects well the variability of hyena diet, a transformation of taphonomic data to quantitative representation of dietary components is only an interpretation, not free from simplifications and uncertainties. Our mean diet model, and we think that any other dietary model based on bone remains, would have the same limitations, which are based on several assumptions: (1) all important dietary components are recorded in the assemblage; (2) proportions of NISPs reflect the proportion of consumed individuals; (3) a bone piece from a taxon reflects a nutrient contribution that is proportional to the taxon's body mass; and (4) body masses of consumed taxa were average body masses for all of the taxa. All these assumptions are simplifications of real situations but are necessary for choosing mathematical inputs for the model. Below we discuss the importance of these assumptions and possible bias they cause.

Assumption 1: all important dietary components are recorded in the assemblage

Because spotted hyenas are obligatory carnivores, we neglected any non-vertebrates as significant food sources. Also, we did not notice in the Perspektywiczna Cave assemblage any missing important dietary component known from other European hyena sites. The analyzed number of bones from the studied taphocenosis was large enough to well represent the assemblage taxonomic variability. Thus, we consider this assumption as acceptable.

Assumption 2: proportions of NISPs reflect the proportion of consumed individuals

At archaeological and paleontological sites, NISP does not always reflect the number of individuals (Lyman, 2008). However, the MNI (minimum number of individuals) approach, which traditionally is based on the bone anatomy (Lyman, 2008), cannot be applied in the case of highly fragmented and anatomically unidentified bone fragments. In this case, NISP offers the best and only representation of the proportion of every individual taxon in the hyena diet. Moreover, due to the long chronology of the Perspektywiczna Cave assemblage, we consider it unlikely that a significant number of bones randomly selected for analysis may come from one individual. It must be noted, that in our model we do not estimate the real amount of consumed prey individuals, but only a proportion between individuals of each taxon. We also expect that future excavations and progress in overall understanding of the assemblage structure will show us if any corrections to our model are necessary.

Assumption 3: a bone piece reflects a nutrient contribution proportionally to the body mass

When NISP provides an estimation of each taxon's quantity in hyena diets, the nutrient contribution from each taxon depends on its body size and offered amount of edible biomass (Hedges and van Klinken, 2002). For example, large-body-mass taxa may account for a small proportion of the faunal assemblage, but their dietary importance can be much greater than that of smaller prey. This is because a single individual, and even a single bone, of a large animal (e.g., rhinoceros or mammoth) represents a much higher amount of consumable biomass than a single item of a smaller animal (e.g., reindeer).

Assumption 4: body masses of consumed taxa were average body masses for all of the taxa

In our model we assume the estimated average body mass of adults. Actual body mass of hyena prey could vary due to body mass differences between sexes, age classes (juveniles, subadults, adults), and animals of different health conditions. Because we were not able to identify the sex, individual age, or health in

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highly fragmented and digested bone material, we used an average adult body mass as the closest approximation. It also must be noted that in our model we do not estimate the real amount of consumed biomass, but only a proportion between biomass from each taxon. Therefore, assuming similar body size variability in all taxa, the proportion reflected by adult body sizes should be still valid even if there were intra-taxa body mass variations.

TDF models

Our four fossil-based models (i.e., assuming hyena bones are included into hyena diet versus excluded from the diet, and with biomass consumed estimation based on Ackerman et al.'s [1984] index [*ABC*] versus simple biomass consumed [*BC*] estimations) gave nearly the same results (Table 3 and Figure 4). Particularly important is the lack of differences in the TDF for models with and without hyena bones themselves included into hyena diet. Perspektywiczna Cave hyenas were certainly cannibalistic to some extent, due to hyena bones being heavily gnawed and digested. Similar situations were widely noticed from Pleistocene hyena den sites across Europe (Hill, 1989; Stiner, 1994, 2004; Palomares *et al.*, 2022) and from modern African hyena dens (Kruuk, 1972). According to ZooMS identification, hyena bones constituted 7% of the digested bones in the Perspektywiczna Cave assemblage; well-preserved and morphologically identifiable hyena bones with gnawing marks were also noticed (Krajcarz *et al.*, 2023). Despite this relatively high amount of hyena remains, the model including hyenas into the diet did not produce a different TDF. One reason for this is the relatively low hyena body mass, which means the hyena's 7% of the total NISP corresponds to only 1% of the total consumed biomass (see Supplementary Information: sheet 1). Even this amount may be overestimated, because hyenas could gnaw and digest old bones of their relatives found in the cave, which accumulated at the site but were not a significant source of their usual diet. Nevertheless, our models imply that cannibalistic behavior in a carnivore, even if clearly recorded in the fossil assemblage, might have limited contribution to the nutrition and thus limited effect on the TDF and the carnivore trophic position.

TDF in hyena and other carnivorans

The Δ^{15}N collagen-to-collagen TDFs that we found for spotted hyena (Table 3) are similar to species-specific collagen-to-collagen TDFs known for other carnivorans (Figure 4). It is within the widely accepted range, around 3–5‰ (Bocherens, 2015; Drucker, 2022), and is within the range inferred from wide population studies (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Schoeninger, 1985; Ambrose and DeNiro, 1986; Van der Merwe, 1989; Schwarcz, 1991; Szepanski *et al.*, 1999; Bocherens and Drucker, 2003; Fox-Dobbs *et al.*, 2007; Krajcarz *et al.*, 2018).

Hyena Δ^{13}C collagen-to-collagen TDFs are clearly higher than in other carnivorans (Figure 4) and above the widely accepted range of around 0.8–1.3‰ (Bocherens, 2015; Drucker, 2022). This discrepancy likely reflects different feeding behaviors (i.e., consumption restricted to soft tissues by other carnivorans versus consumption of both soft tissues and bones by hyena). These two dietary sources, soft tissues and bones, differ in isotopic

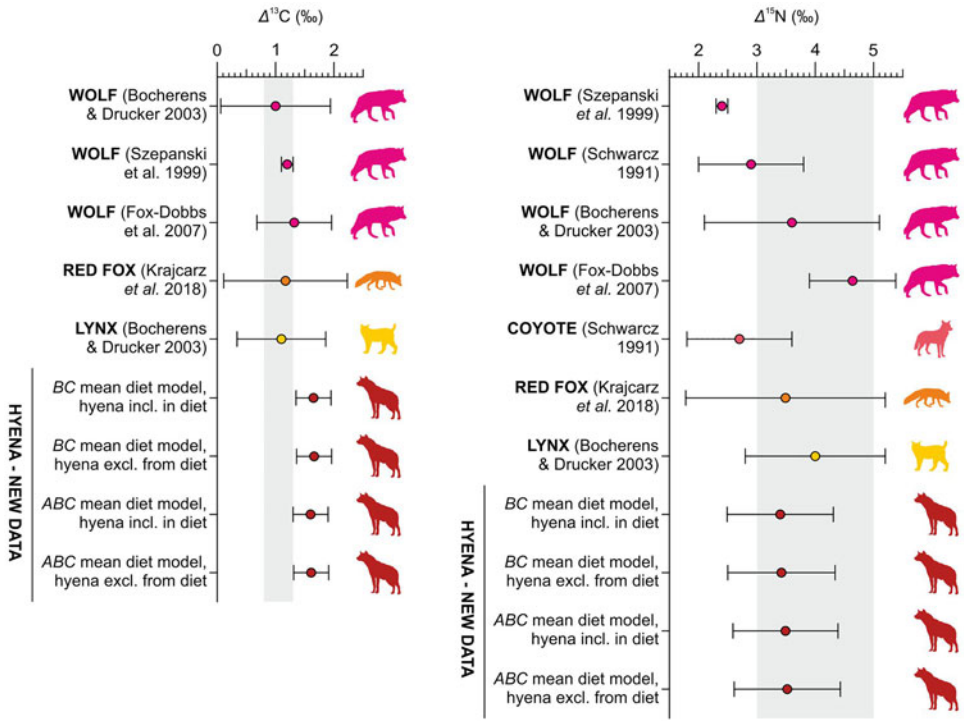

Figure 4. Species-specific collagen-to-collagen TDF (trophic discrimination factor) values (dots) and their 1σ standard errors (whiskers) for terrestrial mammalian carnivores, including our new hyena data. Published data from Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Schoeninger, 1985; Ambrose and DeNiro, 1986; Schwarcz, 1991; Szepanski *et al.*, 1999; Bocherens and Drucker, 2003; Fox-Dobbs *et al.*, 2007; input data are provided in Supplementary Information: sheet 3. The gray bar represents the TDF range usually accepted in paleoecological studies of Quaternary fossil faunas (Bocherens, 2015; Drucker, 2022). *BC* = biomass consumed; *ABC* = biomass consumed using methodology of Ackerman *et al.* (1984) to transform the NISP of each taxon's bone fragments into that taxon's consumed biomass.

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composition of carbon, with $\delta^{13}C$ values of bone collagen exceeding values of other tissues by several per mil (Hobson and Quirk, 2014; Stephens *et al.*, 2023). It is therefore expected that bone-eaters who consume significant amounts of ^{13}C-enriched food would record higher $\delta^{13}C$ signals in their tissues and thus would exhibit higher TDF values.

SIDER versus fossil bone-based TDFs

The TDF from the SIDER model has the largest standard error (Table 4, Figure 5), which, at least for $\Delta^{13}C$, includes the whole range of the fossil-bone-based meat- and hair-related TDFs. The mean value of the SIDER-modeled collagen-diet TDF fits the area of the bone assemblage-based $TDF_{collagen\text{-}meat}$, while all other calculated bone assemblage-based TDFs show lower $\Delta^{13}C$ mean values. For $\Delta^{15}N$, the SIDER model predictions are closer to the calculated TDFs, even if their $\Delta^{15}N$ values are a little bit higher.

To date, there are only two studies that have compared the SIDER model predictions with field studies (Healy *et al.*, 2018; Stephens *et al.*, 2022). In the Stephens *et al.* (2022) study, hair-to-diet TDFs in herbivorous, omnivorous, and insectivorous small mammals were compared. The SIDER models for herbivores and omnivores seemed biased in their $\Delta^{13}C$ prediction, as the calculated diet consistently had higher $\delta^{13}C$ values than those determined by field studies. However, the SIDER predictions for the carnivores (insectivorous shrews) were quite accurate. Furthermore, the SIDER-predicted $\Delta^{15}N$ values were in very good agreement for all species studied. We can also see this trend in our study (Figure 5). As in Stephens *et al.* (2022), the SIDER model led to higher $\Delta^{13}C$ than modeled from the fossil assemblage. The SIDER-predicted $\Delta^{15}N$ values are slightly lower than the bone assemblage-based TDFs.

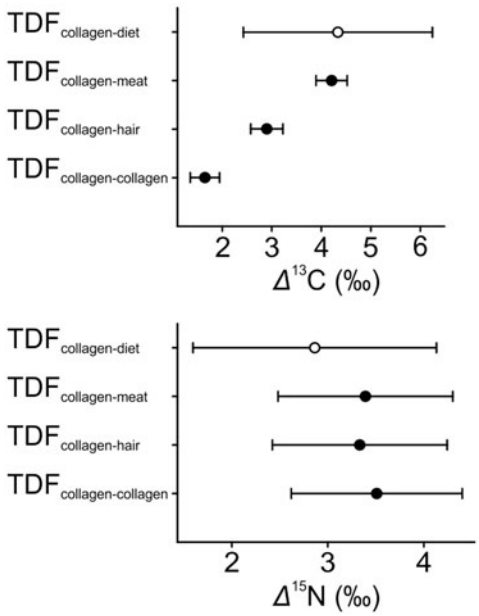

Figure 5. Comparison of the $TDF_{collagen\text{-}diet}$ calculated by the SIDER model (open circle) with a set of collagen-to-other tissues TDFs derived from the Perspektywiczna Cave fossil assemblage (black dots; only the TDFs based on the *ABC* mean diet model with hyena bone excluded from the hyena diet are shown here; see Table 3 for other TDF models, which provided similar values). *ABC* = biomass consumed using methodology of Ackerman *et al.* (1984) to transform the NISP of each taxon's bone fragments into that taxon's consumed biomass; TDF = trophic discrimination factor.

Our comparison shows a small weakness in the SIDER prediction, as the predictions made are only as good as the data on which they are based. The TDF database used by the SIDER algorithm (Healy *et al.*, 2018; Stephens *et al.*, 2022) is based on only 14 mammal and bird species with collagen data. This includes only three carnivorous species (two cetaceans and one charadriiform bird, data from Hobson and Clark, 1992; Toperoff, 2002; Borrell *et al.*, 2012), none of them being closely related to hyenas. Moreover, some of the cetacean data were not true collagen-diet TDFs, but rather collagen-muscle TDFs (Toperoff, 2002). With this limitation in mind, it is positive to note that the SIDER-modeled carbon and nitrogen TDFs are close to the range of our diet-component-specific TDFs.

Conclusions

In this study we provide prey–predator collagen-to-collagen TDFs ($\Delta^{13}C$ and $\Delta^{15}N$) for the Pleistocene cave hyena that were derived from the fossil bone accumulation in Perspektywiczna Cave (Poland). Our results revealed similarity between $\Delta^{15}N$ values for hyena and those previously known for other predators, but clearly higher $\Delta^{13}C$ values in hyena, which are above the widely accepted collagen-to-collagen TDF range in paleoecological studies. This discrepancy is diet-specific and reflects different uptake of nutrients from prey, in particular consumption of bones by spotted hyena. This leads us to suspect that other bone-eaters (e.g., striped hyena, [*Hyaena hyaena*] or wolverine [*Gulo gulo*]) can also have elevated TDF values, which should be considered in paleoecological reconstructions of trophic webs. Moreover, our results clearly indicate that TDF must be established individually for each important species. Our study also revealed that the R package SIDER can provide a provisional approximation of a TDF for mammalian carnivores, but a much wider database of species-specific TDFs is necessary to increase the validity of the SIDER models.

Our study highlights the usefulness of ZooMS analyses in the diet reconstruction of fossil assemblages. It also raised the call for action for more detailed studies combining morphology-based and ZooMS-based taxonomy identification to understand better the formation of bone accumulations related to hunting and scavenging behavior of bone collectors and the body sizes of their prey.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/qua.2024.43.

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Author contributions. MK and MTK: conceptualization; MK: collection curation, bone identification, sampling; MTK, MK, HB: isotopic analysis; SP: ZooMS analysis; MK, MTK, CB: data processing, MTK, CB: illustrations; MK and HB: financing; all authors: manuscript preparation and reviewing.

Data availability statement. All isotopic and ZooMS determinations data are available in the PANGAEA repository: https://doi.org/10.1594/PANGAEA.971340, along with the MALDI ToF spectra files: https://doi.org/10.1594/PANGAEA.971344. The R script for SIDER is provided in the PANDORA repository (CEMP SITE Community, https://www.doi.org/10.48493/ak8a-z502).

Competing interests. The authors declare no competing interest.

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