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Collagen-to-collagen prey-predator isotopic enrichment ($\Delta^{13}C$, $\Delta^{15}N$) in terrestrial mammals - a case study of a subfossil red fox den

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ABSTRACT

Trophic Enrichment Factor (TEF) is the main parameter used in isotopic trophic ecology. TEF values can be derived from specimens subjected to experimental feeding or from free-ranging specimens whose dietary behavior is well monitored, and it can be measured for different tissues of animal body. Direct collagen-to-collagen TEF is a key parameter for fossil material and needs to be well constrained in order to ascertain the reliability of the palaeodietary models. In this paper, we present isotopic results for a subfossil bone accumulation related to red fox (*Vulpes vulpes*) activity, discovered in an abandoned mine in Potok-Senderki (Poland). The objective was to report $\delta^{13}C$ and $\delta^{15}N$ collagen data for red foxes and their prey. These data were used to calculate a prey-predator collagen-to-collagen TEF and provided important information for interpreting stable isotope fractionation in terrestrial food webs. We used different taphonomic indexes to calculate the fox mean diet. The presence of juvenile and adult individuals of fox allowed us to specify the difference in isotopic enrichment according to the age class of the predator. $\Delta^{13}C$ and $\Delta^{15}N$ values calculated here for fox were similar to TEF values presented previously for wolf and lynx, but characterized by wider standard deviation.

1. Introduction

Stable isotope analysis has a number of applications in ecology, one of which is to quantitatively evaluate the contribution of different prey in the diet of predators (e.g., Ben-David et al., 1997; Post, 2002; Bearhop et al., 2004; Urton and Hobson, 2005; Semmens et al., 2009; Boecklen et al., 2011; Kays and Feranec, 2011; Tarroux et al., 2012; Voigt et al., 2014; Codron et al., 2016). For palaeoecology, isotopic tracking using stable isotopes of carbon and nitrogen in bone collagen is increasingly used to investigate the ancient predators diet and their role in past ecosystems (e.g. Bocherens et al., 1995, 1997, 2005, 2011, 2015, 2016; Hilderbrand et al., 1996; Fox-Dobbs et al., 2007; Guiry, 2012; Yeakel et al., 2013; Bocherens, 2015; Wißing et al., 2016). The main parameter used in isotopic trophic ecology is the Trophic Enrichment Factor (TEF, usually denoted as Δ or ε; reflecting the difference in isotopic ratio between consumers tissues and diet). TEF values can be derived from specimens subjected to experimental feeding in which all dietary items can be monitored for their isotopic composition (e.g., DeNiro and Epstein, 1981; Pinnegar and Polunin, 1999; Hobson and Bairlein, 2003; Codron et al., 2011; Ben-David et al., 2012; Voigt et al., 2014) or from free-ranging specimens in which dietary behavior is well-monitored (e.g. Bocherens and Drucker, 2003; Fox-Dobbs et al., 2007; Borrell et al., 2012). TEFs between diet and animals may be measured for different animal body tissues, most of these tissues being usually available only for recent material (such as blood, hair, muscles) (e.g., Hobson and Clark, 1992; Roth and Hobson, 2000; Sponheimer et al., 2003; DeMots et al., 2010; Hobson and Quirk, 2014; Kurle et al., 2014; McLaren et al., 2015). In contrast, most palaeoecological studies dealing with ancient vertebrate specimens analyze bone or teeth, which are the only tissues preserved as fossils of the predators and their prey. However, very few studies of modern vertebrates include bone collagen (Table 1), probably due to sampling difficulties, as certain samples may not be taken on living animals.

Collagen-to-collagen fractionation between carnivores and their prey is not equivalent to diet-to-collagen fractionation derived from direct measurements of prey body tissues. It should be pointed out that collagen-to-collagen TEF is constrained by some assumptions and data limitations. First, it is skewed toward vertebrate prey and ignores plants and invertebrate food items. In the case of plants, neglecting their contribution to isotopic composition of predator tissues is justified by the low protein content of most plants compared to animal food (Phillips and Koch, 2002). Therefore their contribution to the nitrogen

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Table 1
Collagen-to-collagen TEFs (‰) for $\delta^{13}C$ and $\delta^{15}N$ in terrestrial carnivores by different authors.

Prey → predator	$\Delta^{13}C$	$\Delta^{15}N$	Reference
Ungulate prey → lynx	+ 1.1	+ 4.0	Bocherens and Drucker (2003)
Ungulate prey → wolf	+ 1.0	+ 3.6	Bocherens and Drucker (2003)
Moose, beaver → wolf	+ 1.32	+ 4.64	Fox-Dobbs et al. (2007)
Caribou → wolf	+ 1.2	+ 2.4	Szepanski et al. (1999)
Group of herbivores → group of carnivores	~ + 0.6	~ + 2.7	Schoeninger and DeNiro (1984)
Deer → coyote	–	+ 2.7	Schwarcz (1991)
Deer → wolf	–	+ 2.9	Schwarcz (1991)
Group of herbivores → group of carnivores	–	+ 3.2	Schoeninger (1985)
Group of herbivores → group of carnivores	–	+ 3.0 – + 3.5	Minagawa and Wada (1984)
Group of herbivores → group of carnivores	–	+ 4.8 – + 5.7	Ambrose and DeNiro (1986)
Hare, antelope → lynx, jackal	~ + 2	–	Van der Merwe (1989)
Herbivores → human	–	+ 3 – + 5	Hedges and Reynard (2007)

intake of an omnivorous carnivore is likely to be much less important than that of animal foods (Phillips and Koch, 2002; Bocherens, 2015). Invertebrates are more problematic since their bodies contain nitrogen amounts similar to those of vertebrate meat (e.g., Barker et al., 1998). Moreover, collagen-to-collagen TEF takes into account only the bone collagen of prey and ignores other tissues, which might have different isotopic signature (e.g., Tieszen and Fagre, 1993; Crowley et al., 2010; Lecomte et al., 2011).

The direct collagen-to-collagen TEF is, however, a key parameter for fossil material, as bones usually constitute the only type of tissue preserved in fossil sites. For this reason, collagen-to-collagen TEF needs to be well established in order to make palaeodietary models reliable. Uncertainties in the values of TEFs may lead to errors in the calculation of prey proportions and, therefore, efforts should be made to improve the determination of these fractionation factors. For $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen, the collagen-to-collagen TEFs have been provided either as average values or ranges of values (Bocherens and Drucker, 2003; Fox-Dobbs et al., 2007).

Studies of TEF in the wild are difficult, as they require access to both the tissues of the predator and the tissues of its prey, as well as some precise knowledge of the representation of particular prey in the predator's diet. An especially unique and valuable situation is when we have direct access to those prey specimens that were hunted and eaten by a given predator. This situation is rare and usually researchers may only analyze other specimens in the population of prey species than those actually consumed by the analyzed predators. Only occasionally do we find the remains of a predator's long-term diet in its natural environment. The red fox den in Potok-Senderki, Poland (Krajcarz and Krajcarz, 2014), discussed in this paper, is an example of one of this rare finds.

This site offers a unique opportunity for isotopic study of a bone assemblage representing the fox population and the wide spectrum of its prey. Due to the relatively large number of remains, the representation of particular prey species in the predator's diet can be estimated on the basis of bone representation. This provides an opportunity to investigate the bone collagen of predator population together with the bone collagen of specimens that were eaten by the studied predators. In addition, the presence of young and adult individual foxes allows us to study the difference in isotopic enrichment according to the age class of a predator.

The objective of this study is to report $\delta^{13}C$ and $\delta^{15}N$ collagen data for both wild-living red foxes and their prey, as measured on a subfossil bone accumulation. We use these data to calculate a prey-predator collagen-to-collagen TEF. This new data will provide important information for interpreting stable isotope fractionation in terrestrial food webs and will allow more accurate use of isotopic signals measured in fossil mammalian bones to reconstruct ancient trophic relationships.

2. The red fox den

A rich assemblage of bones resulting from red fox (*Vulpes vulpes*) activity was discovered in an abandoned mine in Potok-Senderki (southeastern Poland) in 2009. The detailed taphonomic description of the assemblage was provided by Krajcarz and Krajcarz (2014). The mine was settled by foxes after it was abandoned by miners in the mid-20th century (Gazda and Ruska, 2005). It served as a place where fox cubs spent the first weeks of their life. Adult foxes brought the food for cubs and remains of food have become accumulated in a number of clusters, each likely corresponding to one season of fox breeding activity. The collection of over 600 bones represents a local spectrum of fox prey items (Table 2). This collection allows us to calculate taphonomic parameters, such as the minimum number of eaten individuals

Table 2
List of taxa and age classes present in the bone assemblage from Potok-Senderki.

Taxon	Age	NISP	MNI	weight (g)
Domestic chicken (*Gallus gallus domesticus*)	Adult	175	28	533.5
Domestic chicken (*Gallus gallus domesticus*)	Juvenile	2	1	4
Common pheasant (*Phasianus colchicus*)	Adult	20	9	7.5
Domestic chicken/common pheasant (*Gallus gallus domesticus/Phasianus colchicus*)	Adult	2	1	1
Domestic goose (*Anser anser domesticus*)	Adult	1	1	6
Hooded crow (*Corvus cornix*)	Adult	3	1	5
Hooded crow/rook (*Corvus cornix/frugilegus*)	Adult	3	1	1.5
Common kestrel (*Falco tinnunculus*)	Adult	1	1	1
Red fox (*Vulpes vulpes*)	Adult	24	4	76.5
Red fox (*Vulpes vulpes*)	Subadult	4	2	10
Red fox (*Vulpes vulpes*)	Juvenile	53	8	21
Domestic cat/wildcat (*Felis catus/silvestris*)	Adult	12	3	36
European badger (*Meles meles*)	Adult	3	1	16
European hare (*Lepus europaeus*)	Adult	52	10	167.5
Domestic rabbit (*Oryctolagus cuniculus domesticus*)	Adult	16	4	26
European hare/domestic rabbit (*Lepus europaeus/Oryctolagus cuniculus domesticus*)	Juvenile	25	7	32.5
Roe deer (*Capreolus capreolus*)	Adult	9	3	241
Roe deer (*Capreolus capreolus*)	Juvenile	51	11	154.5
Red deer (*Cervus elaphus*)	Juvenile	3	3	54
Wild boar/domestic pig (*Sus scrofa/domestica*)	Juvenile	6	3	52
Bats (Chiroptera)	–	1	1	0.5
unidentified birds	–	69	–	–
unidentified mammals	–	67	–	–
Total:		602	103	1447

NISP - number of identified specimens; MNI - minimum number of individuals; weight - total weight of dry remains (± 0.5 g).
Taxonomic identifications, NISP and MNI after Krajcarz and Krajcarz (2014).