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Original Article

Intra-tooth stable isotope analysis reveals seasonal dietary variability and niche partitioning among bushpigs/red river hogs and warthogs

Deming Yang[a,b,c,*], Kevin T. Uno[d,e], Thure E. Cerling[b], Ogeto Mwebi[f], Louise N. Leakey[g,h], Frederick E. Grine[h,i], and Antoine Souron[j]

[a]Interdepartmental Doctoral Program in Anthropological Sciences, Stony Brook University, Stony Brook, NY 11794, USA
[b]Department of Geology and Geophysics, University of Utah, Salt Lake City, UT 84112, USA
[c]Division of Anthropology, American Museum of Natural History, New York, NY 10024, USA
[d]Division of Biology and Paleo Environment, Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY 10964, USA
[e]Department of Human Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA
[f]Osteology Section, Department of Zoology, National Museums of Kenya, P.O. Box 40658-00100 Nairobi, Kenya
[g]The Turkana Basin Institute, P.O. Box 24467 – 00502 Nairobi, Kenya
[h]Department of Anthropology, Stony Brook University, Stony Brook, NY 11794, USA
[i]Department of Anatomical Sciences, Renaissance School of Medicine at Stony Brook University, Stony Brook, NY 11794, USA
[j]University of Bordeaux, CNRS, Ministère de la Culture, PACEA, UMR 5199, F-33600 Pessac, France

[*]Address correspondence to Deming Yang. E-mail: dyang@amnh.org.
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Abstract

How animals respond to seasonal resource availability has profound implications for their dietary flexibility and realized ecological niches. We sought to understand seasonal dietary niche partitioning in extant African suids using intra-tooth stable isotope analysis of enamel. We collected enamel samples from canines of red river hogs/bushpigs (*Potamochoerus* spp.) and third molars of warthogs (*Phacochoerus* spp.) in 3 different regions of central and eastern Africa. We analyzed multiple samples from each tooth and used variations in stable carbon and oxygen isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) and covariances between them to infer seasonal dietary changes. We found that most *Phacochoerus* display C_4-dominated diets, while most *Potamochoerus* display C_3-dominated diets. *Phacochoerus* and *Potamochoerus* that co-occur in the same region display no overlap in intra-tooth $\delta^{13}C$, which suggests dietary niche partitioning. They also show diverging $\delta^{13}C$ values as the dry seasons progress and converging $\delta^{13}C$ values during the peak of the rainy seasons, which suggests a greater dietary niche separation during the dry seasons when resources are scarce than during the rainy season. We found statistically significant cross-correlations between intra-tooth $\delta^{13}C$ and $\delta^{18}O$ in most specimens. We also observed a temporal lag between $\delta^{13}C$ and $\delta^{18}O$ in some specimens. This study demonstrates that intra-tooth stable isotope analysis is a promising approach to investigate seasonal dietary niche variation. However, large inter-individual variations in $\delta^{18}O$ at certain localities can be challenging to interpret. Future studies that expand the intra-tooth stable isotope surveys or include controlled feeding experiments will improve its application in ecological studies.

Key words: dietary ecology, niche partitioning, *Phacochoerus*, *Potamochoerus*, seasonality.

The patterns in which a species utilizes seasonal resources have strong implications for its ecology at both the population and the community levels (e.g., Chesson et al. 2004; Tonkin et al. 2017). A significant challenge associated with much of the eastern African ecosystems lies in the dry seasons (or lean season, Perry and Pianka 1997) when food and water resources are limited. Many generalist consumers opportunistically alter their diets in response to the ephemeral nature of resource availability (e.g., Holt 2008; Yang et al. 2008). Extant African suids, including the giant forest hog, the bushpig/red river hog, and the warthogs, are found across Africa and are often categorized as opportunistic feeders (e.g., Rodgers 1984; Kingdon 1988; Treydte et al. 2006; Meijaard et al. 2011). The bushpig/red river hog (*Potamochoerus* spp.) thrives in a variety of habitats with a diverse composition of dietary items that include plant leaves, stems, roots, seeds, fruits, and animal matter (e.g., Leslie and Huffman 2015; Melletti et al. 2017; Seydack 2017). The warthog (*Phacochoerus* spp.) prefers open environments such as wooded grasslands and consumes predominantly grasses (e.g., Rodgers 1984; Treydte et al. 2006; Edossa et al. 2021). While the two taxa have different habitat preferences, their geographic distributions can have substantial overlap in savanna, woodland, and forest edge environments (Melletti et al. 2017; Seydack 2017; de Jong et al. 2023). Under the concept of ecological niche, co-occurring species cannot occupy the same ecological niche without exerting strong competition on each other (Schoener 1974, 1982). Indeed, studies have found that co-occurring herbivores utilize resources in more contrasting ways during the lean season

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to reduce interspecific competition (e.g., Stewart et al. 2002; Kleynhans et al. 2011; Djagoun et al. 2013). As such, one would expect that *Potamochoerus* and *Phacochoerus* display larger dietary niche separation in the dry season than in the rainy season to avoid competition. Another theory regarding seasonally variable resources is optimal foraging, which manifests in that animals tend to consume their preferred foods (rare/patchy, but in high quality) when resources are relatively abundant during the rainy season, and revert to fallback foods (abundant, but in low quality) when resources are scarce during the dry season (Stephens and Krebs 1986; Robinson and Wilson 1998). Since seasonal grass growth and maturation are important sources of variability in forage quality and abundance in wooded savannas (Grunow et al. 1980), one would expect that high-quality grass shoots available in the rainy season (Djagoun et al. 2016; Paine et al. 2018) are preferred foods among bushpigs/red river hogs, and warthogs (e.g., Treydte et al. 2006; Edossa et al. 2021). As such, the two ecological theories outlined above allow us to make specific predictions on the seasonal dietary changes in these common African suids.

Methods in stable isotope ecology have enabled the investigation of temporally explicit dietary records, which allows the comparing of seasonal foraging patterns (e.g., Dalerum and Angerbjörn 2005; West et al. 2006; Codron et al. 2007; Newsome et al. 2007; Djagoun et al. 2016). Stable isotopes of carbon and oxygen in dental enamel have been used to infer changes in diet, vegetation, and hydroclimate at different evolutionary time scales (e.g., Kohn and Cerling 2002; Lee-Thorp 2008; Clementz 2012). The $^{13}C/^{12}C$ ratio can be used to distinguish dietary items that are sourced from either the C_3 or C_4/CAM photosynthetic pathways: in modern lowland tropical habitats, grasses primarily use the C_4 pathway with relatively high $^{13}C/^{12}C$ ratios; while most trees, shrubs, and forbs use the C_3 pathway with relatively low $^{13}C/^{12}C$ ratios (e.g., Tieszen et al. 1979; Ambrose and DeNiro 1986; Farquhar et al. 1989). The $^{18}O/^{16}O$ ratio can be used to inform rainfall seasonality due to the seasonal $^{18}O/^{16}O$ variations in precipitation, and processes that result in low $^{18}O/^{16}O$ in surface waters during the rainy seasons and high $^{18}O/^{16}O$ during the dry seasons (e.g., Dansgaard 1964; Gat 1996; Rozanski et al. 1996; Bowen and Revenaugh 2003). This seasonal variation is subsequently carried over to the animal's body water through various fluxes such as food and drinking (e.g., Bryant and Froelich 1995; Kohn 1996; Kirsanow and Tuross 2011; Green et al. 2018a). Since dental enamel within a tooth is formed incrementally over several months to years (e.g., Kohn et al. 1998; Passey and Cerling 2002; Uno et al. 2020), it can preserve seasonal variations in dietary carbon and environmental water oxygen isotope ratios. As such, stable carbon and oxygen isotope ratios of sequentially sampled dental enamel are excellent tools to study animals' seasonal dietary behavior (e.g., Balasse 2002; Passey, Robinson et al. 2005; Zazzo et al. 2010; Souron et al. 2012; Makarewicz and Pederzani 2017). Intra-tooth stable isotope analysis has been used to investigate dietary responses to seasonal resource abundance and/or quality in the African bush elephant, the hippopotamus, and the giant forest hog (e.g., Passey and Cerling 2002; Cerling and Viehl 2004; Cerling et al. 2006, 2009; Souron et al. 2012; Chritz et al. 2016). It can be an effective tool to study seasonal dietary patterns in suids that have ever-growing lower canines, and in warthogs that have high-crowned third molars (M3s), the enamel of which preserves seasonal dietary and environmental water variations of multiple years (Frémondeau et al. 2012; Yang et al. 2020).

In this study, we sought to better understand the seasonal dietary ecology in bushpigs/red river hogs and warthogs in central and eastern Africa and to answer how these co-occurring species partition their dietary resources seasonally. The seasonal feeding behavior of bushpig/red river hogs can be particularly interesting to ecologists due to their elusive nature (Kingdon 1988; Seydack 2017). It can also provide a framework for interpreting the dietary ecology of other generalist consumers, including extinct ones. We examined the stable carbon and oxygen isotope composition of tooth enamel in bushpigs/red river hogs and warthogs, to infer their dietary habits on a seasonal time scale. With selected individuals, we use intra-tooth isotope profiles to identify rainy seasons and dry seasons based on $^{18}O/^{16}O$ trends and seasonal changes in diet based on $^{13}C/^{12}C$ trends. Following the ecological niche theory, we predicted that bushpigs/red river hogs and warthogs show more diverging dietary niches during the dry season when resources are scarce. We also examined the correlation between intra-tooth $^{13}C/^{12}C$ and $^{18}O/^{16}O$ profiles for each individual, and compared the correlation patterns among individuals and suid species. Following the optimal foraging theory, we predicted that bushpigs/red river hogs and warthogs would consume more grass shoots (high $^{13}C/^{12}C$) in the rainy season (low $^{18}O/^{16}O$), which manifests in negatively correlated intra-tooth carbon and oxygen isotope ratios in *Potamochoerus* and *Phacochoerus*.

Materials and Methods

Specimens

We selected extant African suid specimens from 3 geographic regions of Africa that represent different rainfall regimes (Fig 1, Table 1). The collection localities span from semi-arid shrublands in northern Kenya to the central African rainforests. We collected enamel samples from 2 extant suid genera: *Phacochoerus* and *Potamochoerus*. We sampled 3 *Potamochoerus* and 12 *Phacochoerus* specimens. The specimens came from the mammalian osteological collections at the Osteology Section of the National Museums of Kenya (Samburu National Reserve, $n = 3$), the Turkana Basin Institute (Sibiloi National Park, $n = 3$), and the Kenyan Wildlife Service collected by Dr Thure E. Cerling (Nakuru National Park, $n = 3$), and the American Museum of Natural History in New York City (all other localities, Table 1, $n = 6$). All specimens are part of osteology collections of the respective local authorities from which we obtained permission to conduct stable isotope sampling (see "Acknowledgements" section).

Intra-tooth stable isotope sampling and analysis

We collected enamel samples from the ever-growing lower canines of *Potamochoerus* specimens, and hypsodont M3s of *Phacochoerus* specimens. We obtained multiple samples from a single tooth using a low-speed Dremel® rotary tool with carbide and diamond drill bits. We used a cylindrical diamond bit (2 mm in diameter) to sample canine enamel, and a ball-head diamond bit (1 mm in diameter) to sample M3 enamel. For the lower canines of *Potamochoerus*, we cleaned and sampled the thin enamel layer at 5 mm intervals (Yang et al. 2020). For M3s of *Phacochoerus*, we first removed the cementum

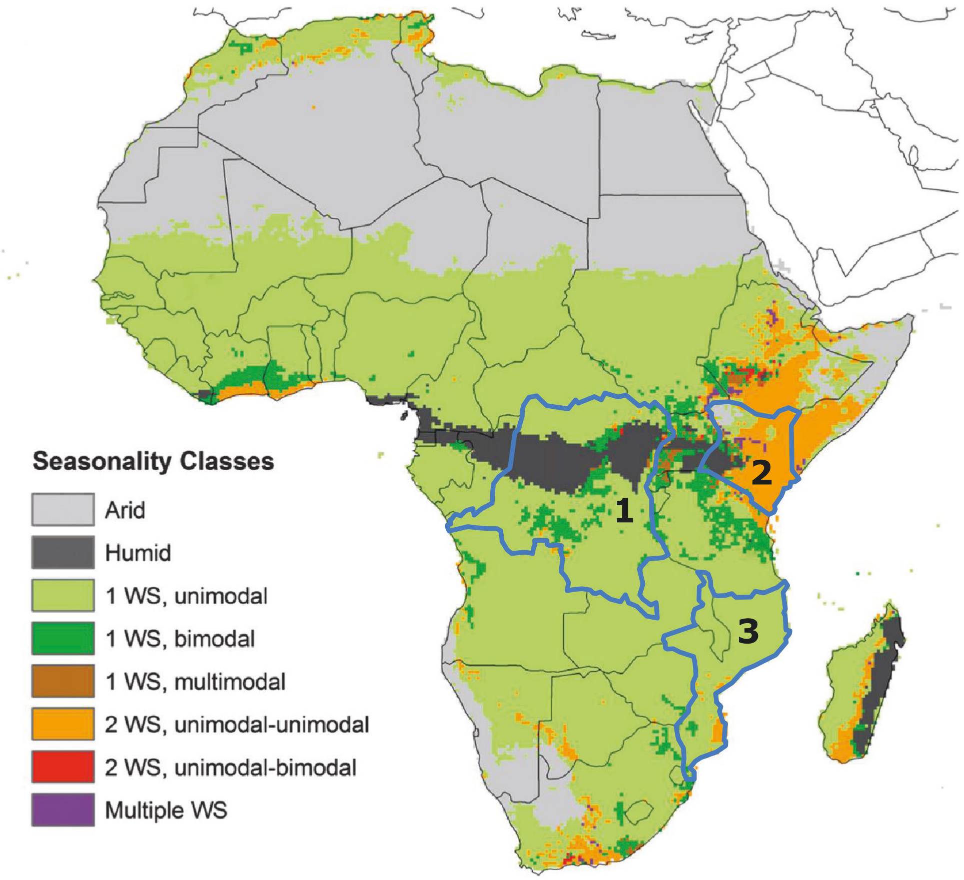

Figure 1. Map of seasonal rainfall patterns (with color-filled pixels) for the continent of Africa, and the geographic origins of the suid specimens investigated in this study (thick outlines, see also Table 1). 1. Democratic Republic of Congo (humid or 1 wet season); 2. Kenya (2 wet seasons); 3. Malawi and Mozambique (1 wet season). Figure modified from Herrmann and Mohr (2011) with permission. WS, wet season.

Table 1. List of extant suids and their associated geographic origin, rainfall regime, biotic zone, and region (for a map view, see Fig 1)

Species	*n*	Locality	Country	Rainfall regime	Biotic zone	Region
Phacochoerus africanus	1	Athi	Kenya	Bimodal rains	Somalia–Masai Bushland	1
Potamochoerus larvatus	1	N/A	Kenya	Bimodal rains	*Somalia–Masai Bushland	1
Phacochoerus africanus	3	Nakuru	Kenya	Bimodal rains	Somalia–Masai Bushland	1
Phacochoerus africanus	3	Sibiloi,	Kenya	Bimodal rains	Somalia–Masai Bushland	1
Phacochoerus aethiopicus	3	Samburu	Kenya	Bimodal rains	Somalia–Masai Bushland	1
Phacochoerus africanus	1	Faradje	DR Congo	Tropical unimodal rains	Northern Rainforest-Savanna Mosaic	2
Potamochoerus porcus	1	Upper Uele	DR Congo	Tropical unimodal rains	Northern Rainforest-Savanna Mosaic	2
Potamochoerus larvatus	1	N/A	Malawi	Unimodal rains	*Zambezian woodland	3
Phacochoerus africanus	1	Inhambane	Mozambique	Unimodal rains	*Zambezian woodland	3

Rainfall seasonality regimes are from Herrmann and Mohr (2011). Biotic zones are from Happold and Lock (2013). Asterisks (*) denote the inferred biotic zone represented by the country of origin. DR Congo, Democratic Republic of Congo.

layer covering the lateral surface of the pillars using a carbide drill bit. Because the outer enamel surface on warthog M3s is rugose and intersects with the inner cementum (Yang et al. 2020), we removed the outer ca. 0.2 mm of enamel to avoid contamination. We then cleaned the surface and sampled the enamel at 2 mm intervals. The target sample mass was 5 mg per sample for both canines and M3s.

We pretreated all enamel samples with 3% hydrogen peroxide and 0.1 M Na-acetate buffered acetic acid for 30 min each with 3 rinses after each step using deionized water, then left to dry at 50 °C overnight. Previous studies have reported ^{18}O-enriched oxygen isotope ratios with the hydrogen peroxide and buffered acetic acid pretreatments, but not for the carbon isotope ratios (e.g., Pellegrini and Snoeck 2016; Spencer et al. 2024). As such, we caution that the oxygen isotope values reported here are potentially ^{18}O-enriched compared to the untreated values. We analyzed the enamel samples at the Stable Isotope Ratio Facility for Environmental Research (SIRFER), University of Utah, where enamel powder samples were reacted with >100% H_3PO_4 at 50 °C in individual glass vials using a Thermo Finnigan GasBench II. The resulting CO_2 was extracted using a GC-TC PAL autosampler connected to a Conflo IV. $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios of the CO_2 were measured using a Thermo Finnigan MAT 253. All isotopic ratios are reported using conventional per mil (‰) notation relative to the Vienna Pee Dee Belemnite (VPDB) standard, where $\delta^{13}C$ or $\delta^{18}O_{sample} = (R_{sample}/R_{standard} - 1)$ (‰); and R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{18}O/^{16}O$ ratios in the sample and standard for $\delta^{13}C$ and $\delta^{18}O$, respectively. We corrected the enamel data using Carrara carbonate and internal laboratory carbonate standards. We corrected $\delta^{18}O$ values with the acid fractionation factor of calcite $\alpha = 1.00930565$, at 50 °C (Passey et al. 2007). A detailed description of the reference materials, calibration method, and the associated instrument biases, and measurement uncertainties is available in the Electronic Supplemental Material. Analytical precision was <0.23 ‰ for $\delta^{13}C$ and <0.27 ‰ for $\delta^{18}O$ (see ESM for details).

Intra-tooth isotopic data comparison

Within an intra-tooth series, we arranged the $\delta^{13}C$ and $\delta^{18}O$ data of enamel samples from the crown apex to the enamel-root junction (close to the cervix of the crown) for molars, or to the open pulp cavity for ever-growing canines, to be consistent with the chronological order of the samples. In each individual, the measured $\delta^{13}C$ values were corrected for the Suess Effect by referencing the $\delta^{13}C$ difference between the preindustrial atmospheric CO_2 at −6.3‰ (Cerling et al. 2015) and that of the collection year of the individual and are reported as $\delta^{13}C_{1750}$ (Supplementary Material, ESM data, Graven et al. 2017). Yang et al. (2020) identified a $\delta^{13}C$ gap between the canine and molar enamel of *Phacochoerus africanus*. A smaller isotopic gap between canine and incisor enamel is present in the wild boar (*Sus scrofa*) with data from Frémondeau et al. (2012), supporting the need to account for any potential isotopic gap between tooth positions in suids (Yang et al. 2020). Therefore, to make dietary inferences of different dental elements comparable, we corrected the $\delta^{13}C$ data of *Potamochoerus* canine enamel using the average $\delta^{13}C$ spacing between molar and canine enamel at −2‰ (Yang et al. 2020). To interpret the percentage of dietary C_4 from $\delta^{13}C_{1750}$ values of each data point, we used a simple linear mixing model. We assumed that the $\delta^{13}C_{1750}$ of C_3 closed canopy, C_3 open canopy, and C_4 end members of mesic biomes are −32.6‰, −26.6‰, and −10.0‰, respectively (Cerling and Harris 1999; Cerling et al. 2015). Since both *Potamochoerus* and *Phacochoerus* are non-coprophagous hindgut fermenters and more closely related to the domestic pig (*Sus scrofa*) than any other taxa with a known apparent isotope enrichment factor between diet and enamel (Cerling et al. 2021), we used the average value of the domestic pig, with $\varepsilon^* = 13.3$‰ (Passey, Robinson et al. 2005), while acknowledging potential variability among individuals (Warinner and Tuross 2009). As such, we obtained 1.4‰ as the end member $\delta^{13}C$ value of molar enamel for 100% C_4 diet, −14.5‰ as the end member $\delta^{13}C_{1750}$ value for 0% C_4 (or 100% open canopy C_3) diet, and −19.7‰ as the end member $\delta^{13}C_{1750}$ value for 100% closed canopy C_3 diet (ESM code). The corrected enamel $\delta^{13}C$ values are plotted against this preindustrial 0–100% dietary C_4 scale, with 25%, 50%, and 75% cutoff lines (see Figures and ESM code).

Due to the complex geometry of enamel mineralization that influences both the shape and the magnitude of the original seasonal signal of $\delta^{18}O$ in the animals' drinking water (e.g., Kohn 1996; Green et al. 2018b) and the lack of an appropriate inverse modeling framework for quantitative interpretation of warthog molar enamel $\delta^{18}O$ data (e.g., Yang et al. 2020), we interpreted enamel $\delta^{18}O$ data primarily with a qualitative approach. Previous studies suggest that both *Phacochoerus* and *Potamochoerus* are highly dependent on accessible drinking waters in their habitats (e.g., White and Cameron 2009; Melletti et al. 2017; Kihwele et al. 2020). Since seasonal changes in $\delta^{18}O$ of drinking water input and the fraction of evaporative water output are buffered by the reservoir effect of the animal's body fluid, the differentials ($d\delta^{18}O/dt$) of body water $\delta^{18}O$ correspond to the water balance of the animal (e.g., Kohn 1996; Green et al. 2018b). Assuming that the differentials ($d\delta^{18}O/dt$) of enamel $\delta^{18}O$ follow those of the body water, for both the bimodal and unimodal rainfall patterns represented by the localities included in this study, we inferred the dry versus rainy seasons using the $\delta^{18}O$ data trend for selected individuals, with positive $d\delta^{18}O/dt$ representing the dry season, while negative $d\delta^{18}O/dt$ representing the rainy season (Yang et al. 2020). We also tested how enamel mineralization may have affected our interpretation of $d\delta^{18}O/dt$ by applying the inverse method (Passey, Cerling et al. 2005) to data of selected individuals (results reported in ESM, see also ESM code) using the R script developed by Britton et al. (2023). We used cross-correlation analysis ("ccf" function, R Core Team 2023) to assess the cross-correlation pattern and any lag between the $\delta^{13}C$ and $\delta^{18}O$ profiles, to potentially identify different forms of seasonal dietary responses (ESM code).

Results

Intra-tooth stable isotope profiles

The preindustrial carbon isotope values ($\delta^{13}C_{1750}$) of extant *Phacochoerus* generally fall within a relatively narrow range between −1‰ and 3‰ (Table 2, Figure 2), which corresponds to a C_4-dominated diet (% dietary $C_4 > 75$, Figure 2), most likely grasses. The only exception among all the extant warthog specimens is P04 from Faradje, DR Congo (Table 3), which drops to −4.3‰ (Table 2), showing a C_3–C_4 mixed diet for parts of the intra-tooth sequence (25 < % dietary $C_4 < 75$, Figure 2). In comparison, intra-tooth $\delta^{13}C_{1750}$ values of *Potamochoerus* are much more variable, generally falling between −15‰ and −5‰. For the P01 specimen (DR Congo), the $\delta^{13}C$ values are consistently below −13.6‰ with little variation (Figure 3), consistent with a C_3 diet of a mixture of open and closed canopy sources. In contrast, P03 and P05 (Malawi and Kenya, respectively) have seasonally variable diets, with parts of the year with predominant C_3 diets (% dietary $C_4 < 25$), and other times with C_3–C_4 mixed diets (25 < % dietary $C_4 < 75$, Figure 3).

Intra-tooth $\delta^{18}O$ values among *Potamochoerus* range from −5‰ to 2‰ (Figure 3). In comparison, intra-tooth $\delta^{18}O$ values among *Phacochoerus* are generally greater than 0‰ except for 2 specimens from Samburu, Kenya, which results in a wide range of $\delta^{18}O$, from −4‰ to 6‰ (Figure 3). For the Nakuru, Samburu, and Sibiloi localities, (Kenya, Table 1), the inter-individual $\delta^{18}O$ range of *Phacochoerus* (highest value-lowest value among intra-tooth $\delta^{18}O$ medians for each locality, Table 3) is 2.1‰, 5.1‰, and 1.1‰, respectively. Intra-tooth $\delta^{18}O$ variations (highest value-lowest value within a profile) in *Potamochoerus* range from 2‰ to 5‰ (Table 3). Results of the inverse modeling method show slightly higher

Table 2. Summary statistics of $\delta^{13}C_{1750}$ in the intra-tooth profiles of this study

Specimen (*n*)	Species	Country	Mean	1σ	Median	Minimum	Maximum	Range	IQR
*P01 (10)	*Po. porcus*	DR Congo	−14.3	0.5	−14.2	−15.0	−13.6	1.4	0.7
*P03 (20)	*Po. larvatus*	Malawi	−9.3	2.5	−9.5	−13.1	−4.8	8.3	4.2
*P05 (19)	*Po. larvatus*	Kenya	−12.1	1.6	−11.9	−14.9	−8.9	6.0	2.4
P04 (15)	*Ph. africanus*	DR Congo	−1.3	1.8	−0.7	−4.3	1.1	5.4	2.3
P06 (17)	*Ph. africanus*	Kenya	0.9	0.8	1.1	−0.7	1.8	2.5	1.1
P07 (17)	*Ph. africanus*	Mozambique	1.65	0.5	1.7	0.6	2.3	1.7	0.5
NKU245 (16)	*Ph. africanus*	Kenya	1.5	0.4	1.6	0.8	2.2	1.4	0.4
NKU257 (11)	*Ph. africanus*	Kenya	1.4	0.2	1.4	1.0	1.8	0.8	0.3
NKU265 (9)	*Ph. africanus*	Kenya	1.7	0.6	1.9	0.2	2.1	1.9	0.3
SAM01 (14)	*Ph. aethiopicus*	Kenya	0.7	0.9	0.5	−0.4	2.1	2.5	1.6
SAM02 (11)	*Ph. aethiopicus*	Kenya	1.3	0.7	1.2	0.3	2.5	2.2	1.1
SAM03 (12)	*Ph. aethiopicus*	Kenya	0.7	1.2	0.9	−1.0	2.0	3.0	2.0
SBL01 (14)	*Ph. africanus*	Kenya	2.1	0.6	2.3	0.4	2.8	2.4	0.6
SBL02 (17)	*Ph. africanus*	Kenya	1.7	0.1	1.6	1.5	1.9	0.4	0.2
SBL03 (16)	*Ph. africanus*	Kenya	1.8	0.3	1.8	1.3	2.2	0.9	0.4

Asterisks indicate specimens (*Potamochoerus* canines) in which the −2‰ carbon isotope correction is applied (Yang et al. 2020). All isotope ratios are expressed with the per mill (‰) notation relative to the Vienna Pee Dee Belemnite (VPDB) standard. *Po.*, *Potamochoerus*; *Ph.*, *Phacochoerus*; IQR, interquartile range; DR Congo, Democratic Republic of Congo.

amplitudes of seasonal variation in the model output than the measured $\delta^{13}C$ and $\delta^{18}O$ profiles (see ESM for more details).

When quasi-sympatric *Phacochoerus* and *Potamochoerus* individuals are plotted together, *Phacochoerus* display consistently higher $\delta^{13}C$ than *Potamochoerus* (Figure 4): there is no overlap in their intra-tooth $\delta^{13}C$ or inferred isotope niches. *Phacochoerus* spp. also display consistently higher $\delta^{18}O$ values than *Potamochoerus* spp.

Cross-correlation between carbon and oxygen isotope intra-tooth profiles

Sixteen out of 24 specimens exhibit significant cross-correlation results between $\delta^{13}C_{1750}$ and $\delta^{18}O$ profiles (Table 4). Among the significantly cross-correlated specimens, 5 specimens show no lag (lag = 0) between $\delta^{13}C$ and $\delta^{18}O$ profiles, while 3 different lag positions (−1, 1, and 3) tie for second place between $\delta^{13}C_{1750}$ and $\delta^{18}O$. Other lag positions (−3, −2, and 2) are much less frequent (Table 4). Among the 16 specimens with significant correlation, one is *Potamochoerus larvatus* (P03) and its $\delta^{13}C_{1750}$ and $\delta^{18}O$ profiles are negatively correlated (Cross-correlation coefficient = −0.743). The other 15 are *Phacochoerus* specimens: 10 display positive cross-correlation coefficients, while 5 display negative cross-correlation coefficients (Table 4). Cross-correlation of the inverse model output shows generally consistent results as that of measured $\delta^{13}C_{1750}$ and $\delta^{18}O$ profiles in both canines and M3s (ESM).

Discussion

Seasonal diets of *Phacochoerus* and *Potamochoerus*

The intra-tooth $\delta^{13}C_{1750}$ results of *Phacochoerus* suggest a predominant C_4 diet in the three studied regions of Africa, despite the diverse vegetation composition represented by the different localities. Since the majority of C_4 plants in Africa are tropical grasses (Cerling and Harris 1999; Codron et al. 2005), our results are consistent with previous studies that suggest warthog teeth are highly specialized and well-adapted to grass consumption (Rodgers 1984; Treydte et al. 2006; Souron 2017; Yang et al. 2022). The seasonal variations in intra-tooth $\delta^{13}C$ are relatively small (typically within 3‰), which can be explained by seasonal variation in the dietary composition of C_4 plants that use different C_4 photosynthetic pathways (NAD/PCK vs NADP) with slightly different $\delta^{13}C$ values (e.g., Cerling and Harris 1999; Codron et al. 2005), or mixing of a seasonally variable but small fractions of C_3 browse and C_3 herbaceous plants into a predominantly C_4 diet. Extant warthogs with a diet dominated by C_3 resources, however, are observed in Malawi (Lüdecke et al. 2016), Nechisar (Levin et al. 2008), and Bale in Ethiopia (Teklehaimanot and Balakrishnan 2017), which is likely due to the predominant C_3 vegetation cover in these localities.

The intra-tooth $\delta^{13}C$ values of *Potamochoerus* are much more variable than those of *Phacochoerus*. The pattern is consistent with results from previous studies that suggest more flexible diets in *Potamochoerus* than in *Phacochoerus* (Melton et al. 1989; Harris and Cerling 2002; Seydack 2017). Specimen P01, which is a *Potamochoerus porcus* (red river hog) specimen, shows a low C_4 diet and low intra-tooth variation. In contrast, specimens P03 and P05, which are *Potamochoerus larvatus* (bushpigs), display highly variable seasonal diets (C_3 feeding to mixed C_3–C_4 feeding) that are consistent with their diverse and likely more heterogeneous habitats (Seydack 2017). These divergent dietary patterns correspond well with their local habitats: the rainforests in DR Congo are dominated by C_3 plants, while the wooded savannas in Malawi and Kenya are a mixture of C_3 and C_4 plants.

The absence of $\delta^{13}C$ overlap among quasi-sympatric *Phacochoerus* and *Potamochoerus* indicates dietary niche partitioning between the two genera. The $\delta^{13}C$ spacing among quasi-sympatric suids is consistently more pronounced in the dry seasons than in the rainy seasons, which

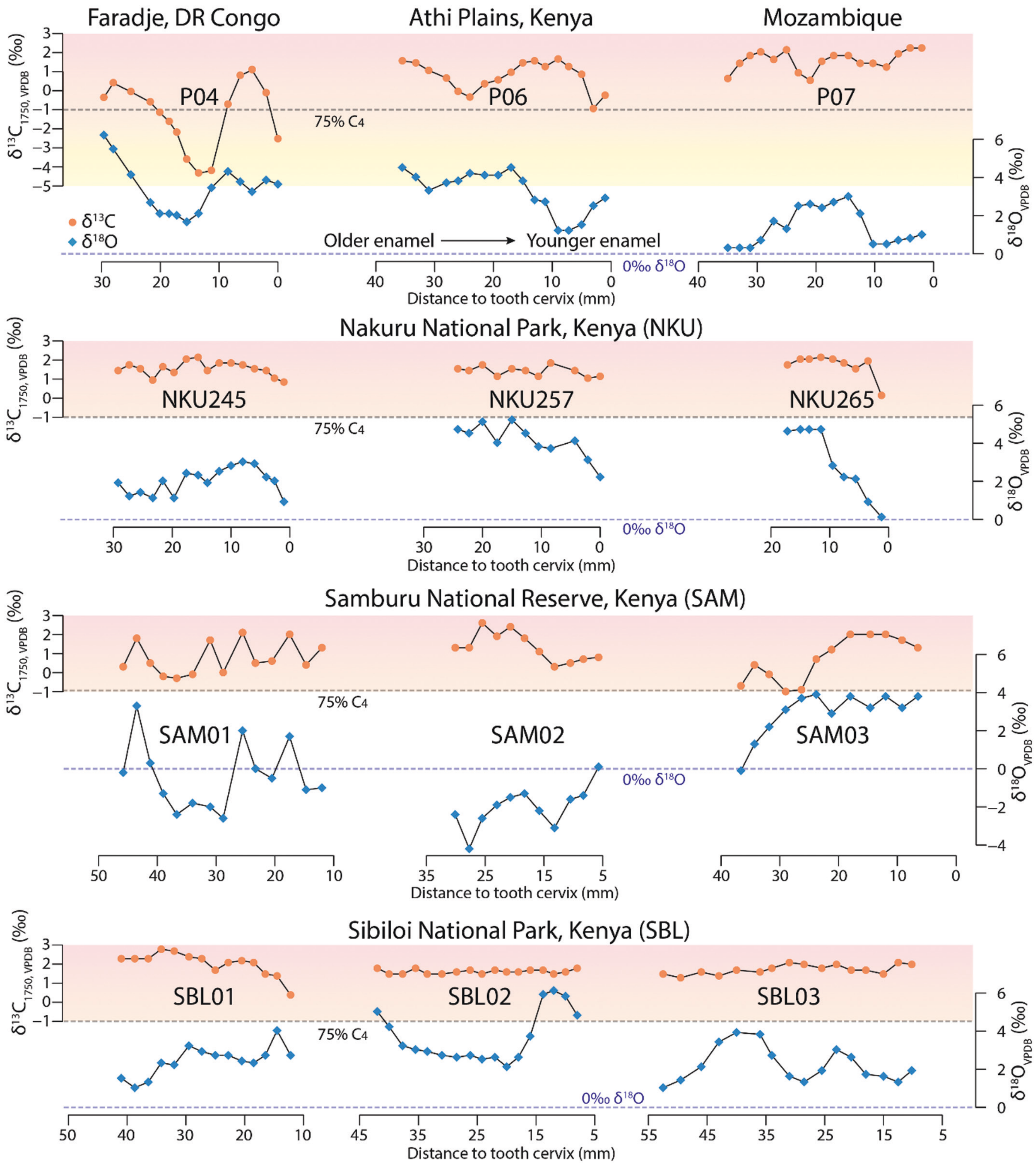

Figure 2. Serial sampling results (filled circles for $\delta^{13}C_{1750}$, filled diamonds for $\delta^{18}O$) of *Phacochoerus* M3s. Gradients indicate nominal % dietary C_4 cutoff levels at horizontal dashed lines. Horizontal dotted lines indicate the $\delta^{18}O$ reference value at 0‰ (VPDB) for comparisons between the panels. VPDB, Vienna Pee Dee Belemnite.

is consistent with our prediction based on the ecological niche theory. One common pattern between the 2 bushpig individuals is that their $\delta^{13}C$ values increased when $\delta^{18}O$ values decreased, indicating an increased consumption of C_4 resources starting in the middle of the rainy seasons and going into the beginning of the dry seasons (Figure 4). This is consistent with our second prediction that bushpigs consume more fresh grass shoots during the rainy seasons. An alternative explanation is seasonal raiding of C_4 crops, such as maize or sugarcane (e.g., Cerling et al. 2006; Nyirenda et al. 2011), as bushpigs are known to cause damage to crops (Melletti et al. 2017). The results suggest that bushpigs are opportunistic feeders that can substantially alter their diets in response to seasonal moisture/food availability (e.g., Breytenbach and Skinner 1982; Melton et al. 1989). Among all the *Phacochoerus* individuals, P04 from Faradje (DR Congo) is the only one that displays a highly variable seasonal $\delta^{13}C$ (Figure 4). The pattern

Table 3. Summary statistics of $\delta^{18}O$ in the intra-tooth profiles of this study

Specimen (n)	Species	Country	Mean	1σ	Median	Minimum	Maximum	Range	IQR
P01 (10)	*Po. porcus*	DR Congo	0.3	0.8	0.0	−0.8	1.8	2.6	0.8
P03 (20)	*Po. larvatus*	Malawi	−0.8	1.2	−0.65	−2.9	1.7	4.6	1.6
P05 (19)	*Po. larvatus*	Kenya	−3.7	0.5	−3.8	−4.3	−2.8	1.5	0.8
P04 (15)	*Ph. africanus*	DR Congo	3.4	1.3	3.5	1.7	6.2	4.5	1.9
P06 (17)	*Ph. africanus*	Kenya	3.2	1.1	3.7	1.2	4.5	3.3	1.4
P07 (17)	*Ph. africanus*	Mozambique	1.4	1.0	1.0	0.3	3.0	2.7	1.9
NKU245 (16)	*Ph. africanus*	Kenya	2.0	0.7	2.0	0.9	3.0	2.1	1.1
NKU257 (11)	*Ph. africanus*	Kenya	4.1	0.9	4.1	2.2	5.2	3.0	0.9
NKU265 (9)	*Ph. africanus*	Kenya	3.0	1.8	2.8	0.1	4.7	4.6	2.6
SAM01 (14)	*Ph. aethiopicus*	Kenya	−0.4	1.7	−0.75	−2.6	3.3	5.9	1.9
SAM02 (11)	*Ph. aethiopicus*	Kenya	−2.0	1.1	−1.9	−4.2	0.1	4.3	1.1
SAM03 (12)	*Ph. aethiopicus*	Kenya	2.9	1.2	3.2	−0.1	3.9	4.0	1.1
SBL01 (14)	*Ph. africanus*	Kenya	2.4	0.8	2.55	1.0	4.0	3.0	0.5
SBL02 (17)	*Ph. africanus*	Kenya	3.7	1.4	3.0	2.1	6.1	4.0	2.2
SBL03 (16)	*Ph. africanus*	Kenya	2.2	0.9	1.9	1.0	3.9	2.9	1.2

All isotope ratios are expressed with the per mill (‰) notation relative to the Vienna Pee Dee Belemnite (VPDB) standard. *Po.*, *Potamochoerus*; *Ph.*, *Phacochoerus*; IQR, interquartile range; DR Congo, Democratic Republic of Congo.

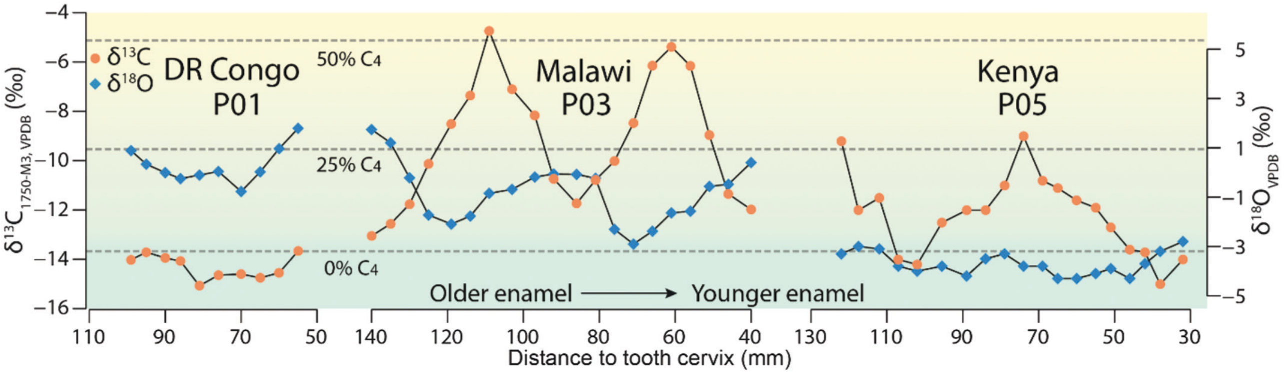

Figure 3. Serial sampling results (filled circles for $\delta^{13}C_{1750}$, filled diamonds for $\delta^{18}O$) of *Potamochoerus* canines. $\delta^{13}C_{1750}$ data are corrected with a − 2‰ carbon isotope spacing between molar and canine enamel (the -M3 subscript, Yang et al. 2020). Gradients indicate nominal % dietary C_4 cutoff levels at horizontal dashed lines. VPDB, Vienna Pee Dee Belemnite.

of its increased C_3 consumption during the rainy season is inconsistent with our second prediction that warthogs consume more grass shoots during the rainy season, even though such a behavior has been observed in more open habitats (e.g., Treydte et al. 2006; Edossa et al. 2021). The extensive woody cover in the local habitat of a rainforest margin and the possibility of opportunistic foraging on desirable C_3 food items/C_3 grass at this locality may explain this unique pattern. On the other hand, most warthogs included in this study show higher $\delta^{13}C$ during the dry seasons, which could be explained by an increased proportion of grass roots (Kingdon 2015) as a fallback food in their diets. Since $\delta^{13}C$ data can only provide information on the general C_3-C_4 spectrum, and not on the types of plant (e.g., C_3 trees, shrubs, and herbaceous plants) or plant parts (e.g., leaves, stems, and roots) in the diet, we think these data may not provide sufficient information to distinguish preferred versus fallback foods. Future studies that combine field observations, tracking, analysis of fecal samples, and expanded isotopic datasets can help to reveal more nuances in the seasonal dietary behaviors in these African suids and potentially inform wildlife management strategies. Nevertheless, our data show that dietary plasticity may be observed in *Phacochoerus africanus*, even though its dentition suggests a highly specialized grassy diet (e.g., Souron 2017; Yang et al. 2022).

$\delta^{18}O$ variations

Phacochoerus display consistently higher $\delta^{18}O$ values than *Potamochoerus*, which is consistent with previous studies that analyzed bulk enamel samples (Harris and Cerling 2002; Lazagabaster et al. 2021). The higher $\delta^{18}O$ values in *Phacochoerus* suggest that warthogs are likely less dependent on drinking water than bushpigs/red river hogs. The higher $\delta^{18}O$ values may also come from leaf water that is ingested with foods. Because C_4 grasses commonly exhibit higher leaf water $\delta^{18}O$ than C_3 dicots due to differences in rooting depth and leaf anatomy (Sala et al. 1989; Dodd et al. 1998; Helliker and Ehleringer 2000; Ogée et al. 2007), grass-feeding *Phacochoerus* spp. can also incorporate the higher $\delta^{18}O$ into

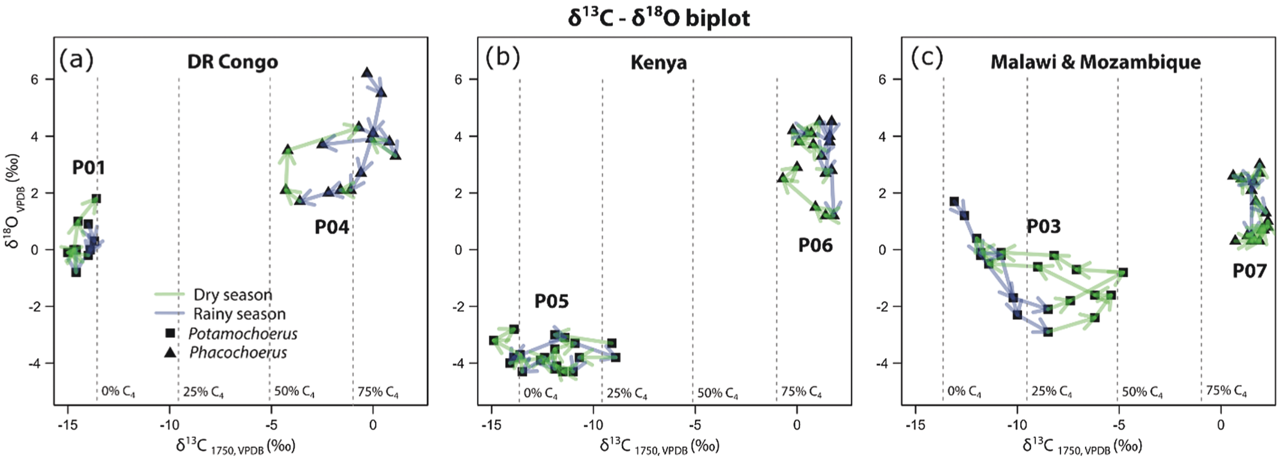

Figure 4. $\delta^{13}C_{1750}$-$\delta^{18}O$ biplots demonstrating overall lack of isotopic overlap between quasi-sympatric individuals of *Potamochoerus* (black-filled squares) and *Phacochoerus* (black-filled triangles) in the 3 geographic regions of interest: (A) Democratic Republic of Congo (DR Congo), (B) Kenya, and (C) Malawi-Mozambique (see also Figure 1). Shaded arrows indicate the chronological sequence of intra-tooth samples and interpretation of rainfall seasonality based on data trends of enamel $\delta^{18}O$ (light-shaded arrows for the dry season, dark-shaded arrows for the rainy season). Vertical dashed lines indicate the nominal % dietary C_4 cutoff levels. VPDB, Vienna Pee Dee Belemnite.

their enamel. The overall intra-tooth $\delta^{18}O$ variations are consistent with expectations for seasonal changes in meteoric water composition in extant suids of central and eastern Africa (Reid et al. 2019; Yang et al. 2020). On the other hand, we noticed large inter-individual variations among *Phacochoerus* from Samburu (SAM01-03, Table 3), where the median enamel $\delta^{18}O$ values between individuals vary between −1.9‰ and 3.2‰ (Table 3). In comparison, inter-individual $\delta^{18}O$ variations among *Phacochoerus* from Nakuru and Sibiloi are much smaller (Table 3). The pronounced inter-individual $\delta^{18}O$ variation found in Samburu could suggest the presence of different water sources (e.g., rivers vs. an evaporated pond) on the landscape, but we do not have sufficient information to validate this.

Cross-correlation between intra-tooth $\delta^{13}C$ and $\delta^{18}O$ profiles

The majority (15 out of 21) of extant *Phacochoerus* specimens show significant cross-correlations between intra-tooth $\delta^{13}C$ and $\delta^{18}O$ profiles. While the consistently high $\delta^{13}C$ values of *Phacochoerus* M3s suggest predominant C_4 diets, cross-correlation analysis provides nuances in the seasonal dietary responses of different individuals. Intriguingly, there seem to be divergent dietary responses to rainfall seasonality between *Phacochoerus* individuals from Nakuru and Samburu with generally positive cross-correlation coefficients, and those from Sibiloi and Mpala with generally negative cross-correlation coefficients (Table 4), despite having a similar rainfall regime among the localities (Figure 1). Such divergent seasonal dietary responses could be related to the local vegetation composition or interspecific competition in their respective feeding guilds. These data further support the dietary plasticity observed in warthogs. Future studies may consider investigating individual niches within a population or among localities, to evaluate resource use patterns among individuals, as well as inter- and intra-specific competition (e.g., Vander Zanden et al. 2010).

The observed "temporal lag" in intra-tooth $\delta^{13}C$ values relative to $\delta^{18}O$ by 1–3 data points is observed in specimens P03, P04, and P06 (Figures 2 and 3). Such a delay in the dietary change has been observed in elephants and horses (Cerling et al. 2009; de Winter et al. 2016), which could be explained by a behavioral response latency between season initiation and changes in plant growth, abundance, and subsequently, animal foraging patterns. Another explanation is the different time scales of physiological turnover for $\delta^{13}C$ and $\delta^{18}O$ within the body of the animal (e.g., Ayliffe et al. 2004; Podlesak et al. 2008), or within the enamel itself during the multi-phase mineralization process (Trayler and Kohn 2017). We consider the former explanation more likely due to the lack of a consistent lag position between the intra-tooth $\delta^{13}C$ and $\delta^{18}O$ profiles, but more data are needed to validate these explanations. Future studies should consider the possibility of a "temporal lag" between intra-tooth $\delta^{13}C$ and $\delta^{18}O$ profiles, and use cross-correlation analysis in the interpretation of seasonal dietary response.

Limitations

Intra-tooth stable isotope analysis is a compelling method for inferring seasonal dietary behavior, but the approach is not without limitations. First, our diet-enamel carbon isotope enrichment factor is referenced from one of the few studies available (Passey, Robinson et al. 2005), which may vary among species depending on the digestive physiology of the animal, or among individuals depending on their dietary composition and/or dietary quality (e.g., Warinner and Tuross 2009; Codron et al. 2011, 2018; Cerling et al. 2021). Another factor that may come into play is stable isotope routing. For example, fasting during the dry season (lean season) may lead to more ^{13}C-depleted signals in enamel due to potentially increased catabolism of stored fat, which is more ^{13}C-depleted than dietary carbohydrates (e.g., O'Brien 2015). These factors are still poorly understood and may introduce uncertainty in studies of inter-specific comparisons of diet and seasonal behavior such as this one. Finally, our study is limited by the small sample sizes included at each study locality, due to the low availability of specimens from the regions. The small sample sizes make it challenging to infer inter-individual $\delta^{18}O$ variations or $\delta^{18}O$ variations between localities, which begs for more detailed investigations.

Table 4. List of extant suid specimens, their locality, dental element, and source of data used in the cross-correlation analysis, and results of cross-correlation analysis between the $\delta^{13}C_{1750}$ and $\delta^{18}O$ profiles among specimens

Specimen	Species	Locality	Element	Dominant lag position	Cross-correlation coefficient	Statistical significance	Data source
P01	*Po. porcus*	Upper Uele, DR Congo	Lower Canine	−1	0.589	NS	This study
P03	*Po. larvatus*	Malawi	Lower Canine	−1	−0.745	S	This study
P05	*Po. larvatus*	Kenya	Lower Canine	−3	−0.395	NS	This study
P04	*Ph. africanus*	Faradje, DR Congo	Lower M3	−1	0.729	S	This study
P06	*Ph. africanus*	Big Tree Camp, Athi plains, Kenya	Lower M3	−2	0.505	S	This study
P07	*Ph. africanus*	Rio Save, 212 km. SSW. Beira, Zinave, Mozambique	Lower M3	−2	−0.348	NS	This study
NKU245	*Ph. africanus*	Nakuru National Park, Kenya	Lower M3	0	0.610	S	This study
NKU257	*Ph. africanus*	Nakuru National Park, Kenya	Lower M3	0	0.547	S	This study
NKU265	*Ph. africanus*	Nakuru National Park, Kenya	Lower M3	0	0.686	S	This study
SAM01	*Ph. aethiopicus*	Samburu National Reserve, Kenya	Upper M3	0	0.693	S	This study
SAM02	*Ph. aethiopicus*	Samburu National Reserve, Kenya	Upper M3	3	−0.389	NS	This study
SAM03	*Ph. aethiopicus*	Samburu National Reserve, Kenya	Lower M3	3	0.720	S	This study
SBL01	*Ph. africanus*	Sibiloi National Park, Kenya	Lower M3	1	−0.682	S	This study
SBL02	*Ph. africanus*	Sibiloi National Park, Kenya	Lower M3	−1	0.326	NS	This study
SBL03	*Ph. africanus*	Sibiloi National Park, Kenya	Lower M3	3	0.375	NS	This study
MPL1M	*Ph. africanus*	Mpala, Laikipia, Kenya	Lower M3	1	−0.847	S	1
MPL2M	*Ph. africanus*	Mpala, Laikipia, Kenya	Lower M3	−2	−0.745	S	1
MPL1C	*Ph. africanus*	Mpala, Laikipia, Kenya	Lower Canine	−1	−0.466	S	1
MPL2C	*Ph. africanus*	Mpala, Laikipia, Kenya	Lower Canine	1	−0.605	S	1
B119	*Ph. africanus*	Naivasha, Kenya	M3	3	0.782	S	2
B384	*Ph. africanus*	Naivasha, Kenya	M3	0	−0.361	NS	2
B58.2	*Ph. africanus*	Naivasha, Kenya	M3	3	−0.439	S	2
B33	*Ph. africanus*	Naivasha, Kenya	M3	1	0.274	NS	2
B56.1	*Ph. africanus*	Naivasha, Kenya	M3	0	0.554	S	2

Dominant lag position is assessed by the highest cross-correlation coefficient values within the lag window of ±3. Statistical significance is assessed by comparing the cross-correlation coefficients with the approximate 95% confidence interval in each specimen (ESM Supplementary Figures S1–S3). Data source 1: Yang et al. (2020). Data source 2: Reid et al. (2019). DR Congo, Democratic Republic of Congo; S, statistically significant; NS, not statistically significant.

Implications for paleoecological studies

The recorded annual to sub-annual variations of $\delta^{13}C$ and $\delta^{18}O$ suggest that dietary response to rainfall seasonality can be inferred from intra-tooth stable isotope analysis of canines or hypsodont molars. Since dental enamel preserves carbon and oxygen isotopic signatures through evolutionary time scales (e.g., Uno et al. 2011; Cerling et al. 2015), this approach can be used to investigate the dietary response to seasonality in extinct taxa (e.g., Higgins and MacFadden 2009; Souron et al. 2012; DeSantis et al. 2017). It has great potential for studying seasonal dietary niche partitioning patterns among sympatric/quasi-sympatric species, and dietary niche flexibility/fidelity of different lineages through time. In the meantime, challenges remain in the interpretation of seasonal cycles from enamel $\delta^{18}O$ results: there is still a lack of consensus on the "best practice" workflow of interpreting intra-tooth $\delta^{18}O$ results (e.g., Pederzani and Britton 2019; Norwood et al. 2023, see also ESM). For example, our interpretation of seasonal cycles is entirely based on the intra-tooth $\delta^{18}O$ trends, which are admittedly associated with assumptions, uncertainties, and potential biases that are yet to be tested or characterized. Other factors that can influence our interpretation include the shape and amplitude of measured intra-tooth $\delta^{18}O$ series, and the lack of data on enamel growth and mineralization patterns in most fossil species. Enamel histology and modeling approaches are common ways to incorporate enamel growth and mineralization information into the interpretation of intra-tooth isotope series (e.g., Passey and Cerling 2002; Passey, Cerling et al. 2005; Green et al. 2018b; Uno et al. 2020; Yang et al. 2020; Britton et al. 2023), but they require substantially more efforts and expertise. The observed temporal lag in intra-tooth $\delta^{13}C$ relative to $\delta^{18}O$ may introduce further complexity to the modeling and interpretation workflow of dietary seasonality from enamel. As such, to better test the assumptions and characterize the uncertainty and biases mentioned above, future studies should consider controlled feeding experiments that investigate how seasonal environmental factors (e.g., temperature and humidity), diet (e.g., nutritional content, digestibility, and transit time), and physiology (e.g., activity level and fasting) can influence the $\delta^{13}C$ and $\delta^{18}O$ variations in enamel.

Conclusions

In this study, we investigated intra-tooth stable isotope variation in ever-growing canines of red river hogs/bushpigs and hypsodont third molars of warthogs in central and eastern

Africa. We found that warthogs generally display a seasonally stable C_4-dominated diet. In comparison, bushpigs display seasonally variable diets with increased C_4 consumption during the rainy season. Quasi-sympatric *Phacochoerus* and *Potamochoerus* show clear dietary niche partitioning. They tend to display a wider dietary niche separation during the dry season, which is consistent with our predictions according to the ecological niche theory. At the same time, 2 out of 3 *Potamochoerus* individuals display higher $\delta^{13}C$ values during the rainy season, while most of the *Phacochoerus* individuals display lower $\delta^{13}C$ values during the rainy season. We think that such information is inadequate to evaluate our predictions based on the optimal foraging theory. Our results suggest that intra-tooth stable isotope analysis can inform seasonal dietary variations for species with relatively flexible diets, or seasonal patterns of resource utilization of co-habiting species. We demonstrate the utility of cross-correlation analysis to evaluate the temporal lag between intra-tooth $\delta^{13}C$ and $\delta^{18}O$ profiles and to inform seasonal dietary response patterns between individuals and we recommend this approach for other studies employing serial sampling of teeth. Our findings suggest that intra-tooth isotope sampling is a promising approach to infer seasonal dietary niche variation in extinct species with hypsodont teeth or continuously growing tusks. Future studies would benefit from larger sample sizes and more detailed surveys on the dietary behavior of these suids, or controlled feeding experiments that help to test specific assumptions associated with this study.

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Authors' Contributions

D.Y. and K.T.U. formulated the research idea. D.Y., K.T.U., T.E.C., O.M., and L.N.L. conducted the fieldwork. D.Y. performed the experiments. D.Y. and K.T.U. analyzed the data. T.E.C., L.N.L., F.E.G., and A.S. provided funding and resources. D.Y. wrote the first draft of the manuscript. All other authors provided critical editorial advice to subsequent drafts and approved the final version for publication.

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Conflict of Interests

None declared.

Ethical Approval

For this type of study, formal ethics approval is not required.

Data Availability

All data associated with this study are part of the dissertation research by Yang (2020) and are available on GitHub via https://github.com/Deming-Yang/SuidDiet. Additional information is available in the Supplementary Material.

Code Availability

Custom R code associated with this study is available on GitHub via https://github.com/Deming-Yang/SuidDiet.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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