SPECIAL TOPIC

CrossMark

Stable isotope ecology of black rhinos (*Diceros bicornis*) in Kenya

Thure E. Cerling[1,2] · Samuel A. Andanje[3] · Francis Gakuya[3] · John M. Kariuki[3] · Linus Kariuki[3] · Jackson W. Kingoo[3] · Cedric Khayale[3] · Isaac Lekolool[3] · Anthony N. Macharia[4] · Christopher R. Anderson[1] · Diego P. Fernandez[1] · Lihai Hu[1] · Shawn J. Thomas[1]

Abstract

Stable isotope and elemental ratios in hair are influenced by the environment, including both climate and geology. Stable carbon isotopes can be used to give estimates of the C_4/CAM fraction of diets of herbivorous mammals; stable nitrogen isotopes are related to the local water deficit; strontium isotopes are determined by the local geology. We studied hair from rhinos in Kenya to determine spatial patterns in $\delta^{13}C$, $\delta^{15}N$, and $^{87}Sr/^{86}Sr$ ratios. The samples of rhino hair were collected during Kenya Wildlife Service translocation or veterinary activities. $\delta^{13}C$ values showed diets dominated by C_3 foods, but in some regions the diet, at least seasonally, contained significant quantities (i.e., > ca. 20%) of C_4/CAM foods. $\delta^{15}N$ values were related to water deficit, with higher $\delta^{15}N$ values in regions with high water deficit. $^{87}Sr/^{86}Sr$ isotope ratios were found to be related to the local geological substrate suggesting that $^{87}Sr/^{86}Sr$ isotope ratios are provisionally useful for determining the origins of illegal wildlife materials in Kenya and elsewhere in Africa.

Samuel A. Andanje: Deceased.

Communicated by Joy K. Ward.

✉ Thure E. Cerling
thure.cerling@utah.edu

Francis Gakuya
gakuya@kws.go.ke

John M. Kariuki
jkariuki@kws.go.ke

Linus Kariuki
lkariuki@kws.go.ke

Jackson W. Kingoo
jwkingoo@yahoo.com

Cedric Khayale
cedric@kws.go.ke

Isaac Lekolool
lekolool@kws.go.ke

Anthony N. Macharia
macharia.anthony@ku.ac.ke

Christopher R. Anderson
christopher.r.anderson88@gmail.com

Diego P. Fernandez
diego.fernandez@utah.edu

Lihai Hu
lihai.hu@utah.edu

Shawn J. Thomas
thomas.j.shawn@gmail.com

[1] Department of Geology and Geophysics, University of Utah, Salt Lake City, UT 84112, USA

[2] Department of Biology, University of Utah, Salt Lake City, UT 84112, USA

[3] Kenya Wildlife Service, PO Box 40241-00100, Nairobi, Kenya

[4] Department of Geography, Kenyatta University, PO Box 43844-00100, Nairobi, Kenya

Introduction

Stable isotope analyses of animal tissues provide important information about the diets of herbivorous mammals (Cerling et al. 2003; Sponheimer et al. 2003), even in the absence

of direct observations. In some cases, regional dietary differences can be determined (van der Merwe et al. 1988) and these can be further used in forensics investigations of wildlife products (e.g., ivory, van der Merwe et al. 1990; Cerling et al. 2007b).

Stable carbon isotopes are very useful in estimating the fraction of C_3 plants versus C_4 plants in the diet of herbivorous mammals. This is because the C_3 pathway has, on average, significantly lower $^{13}C/^{12}C$ ratios than do C_4 plants. C_3 plants comprise trees, shrubs, forbs, and herbs. C_4 plants in East Africa are primarily grasses or sedges, although some C_4 dicots are present in East Africa (for example, *Blepharis*). In Africa, the isotope ratio of CAM plants is generally similar to that of C_4 plants. Some CAM plants (e.g., *Sansevieria*, *Euphorbia*, and *Salsola*) are known to be eaten by black rhinos (Oloo et al. 1994; Ganqa et al. 2005; Lieverloo et al. 2009; Buk and Knight 2010). Because stable isotopes do not distinguish between C_4 plants and CAM in Africa, we will refer to C_4 plants and CAM plants collectively as C_4/CAM for the purposes of rhino isotope ecology.

Likewise, $^{15}N/^{14}N$ ratios are inherited from diet but the interpretation of dietary source is not as straightforward as in carbon because of variation in both soil nitrogen and in N-fixation in plants. *Acacia* are legumes and some are known to fix nitrogen from the atmosphere. However, stable isotope analyses of *Acacia* from Kenya show that many *Acacia* do not fix nitrogen based on their nitrogen isotope ratios. Other important potential nitrogen-fixing plants in rhino diet include *Indogofera*.

$^{87}Sr/^{86}Sr$ isotopes differ in geological regions due to the decay of ^{87}Rb over time; $^{87}Sr/^{86}Sr$ from old geological terrains, especially granitic terrains have high amounts of radiogenic ^{87}Sr, whereas young basaltic rocks, such as are found in the Rift Valley, have very little radiogenic ^{87}Sr. $^{87}Sr/^{86}Sr$ ratios of very young basaltic rocks (i.e., Quaternary) are generally about 0.703–0.705; very old terrains, such as in South Africa may have $^{87}Sr/^{86}Sr$ ratios greater than 0.720. van der Merwe et al. (1990) and Vogel et al. (1990) both proposed that $^{87}Sr/^{86}Sr$ isotope ratios could provide a means to determine the origin of elephant ivory and thus could be a good indicator of wildlife origins. In East Africa, Koch et al. (1995) and Coutu et al. (2016) published $^{87}Sr/^{86}Sr$ isotope ratios for elephant bone and ivory from some known locations. Thus, strontium isotopes in diet are determined primarily by the local geological substrate; however, Graustein and Armstrong (1983), Quade et al. (1995), and Vitousek et al. (1999) showed that the local geological substrate is modified by atmospheric import of dust so that the ecologically available strontium, expressed as $^{87}Sr/^{86}Sr$ ratios, is a combination of the local geological substrate and imported dust. Sr isotope ratios have proven to be important ecological tracers in studying fish ecology (e.g., Kennedy

et al. 1997, 2000; Brennan et al. 2015a, b); bird migration (Blum et al. 2001); and paleoecology (Hoppe et al. 1999).

In this study we report the results of keratin samples (hair) from black rhinos (*Diceros bicornis*) from Kenya that were obtained between 2008 and 2012. We compare isotope ratios of strontium, carbon, and nitrogen from different regions within Kenya that have differing geological substrates and different ecological conditions related to an aridity cline; we also study single hairs for carbon and nitrogen isotopes to understand seasonal diet changes. These regional results suggest that isotopes would be useful in distinguishing the origins of illegal wildlife products, such as rhino horn. We also report the results for carbon and oxygen from tooth enamel from rhinos that died principally during the late 1960s drought in East Africa.

Methods

Sample collection

Hair samples from black rhinos (*Diceros bicornis*) were collected by KWS staff between 2008 and 2012 during translocation, ear-notching, transmitter installation, or treatment procedures between 2008 and 2012. Hair was collected from the tail by plucking so that the proximal end was preserved. Figure 1 shows the localities sampled with the local bedrock geology. Appendix SI 1 gives information about individual rhinos (age, sample collection date, residential history, etc.).

Plant samples were collected from the Laikipia region, the Nairobi region, and from Tsavo East NP between 1997 and 2007. Plants known to be within 50 m of known historic bomas (thorn-bush corrals made by local pastoralists) are not included in this report. C_3 and C_4 plants were identified in the field. Details of the rhino program in Kenya are provided by Western (1982), Oloo et al. (1994), Muya and Oguge (2000), Walpole et al. (2001), Birkett (2002), Walpole (2002), Amin et al. (2006), Patton and Jones (2007), Patton et al. (2007, 2008), Okita-Ouma et al. (2008, 2010), Dharani et al. (2009), Ngene et al. (2011), and Emslie (2013).

Teeth were sampled from the Tsavo East Research Center, using the collections from animals that died in the great drought of the late 1960s (Spinage 1994). Sampled species included the black rhino, elephant (*Loxodonta africana*), giraffe (*Giraffa camelopardalis*), and zebra (*Equus burchelli*), with the three latter species being analyzed for diet comparisons to black rhinos.

Treatment

Hair samples were washed with chloroform–methanol to remove organic contaminants.
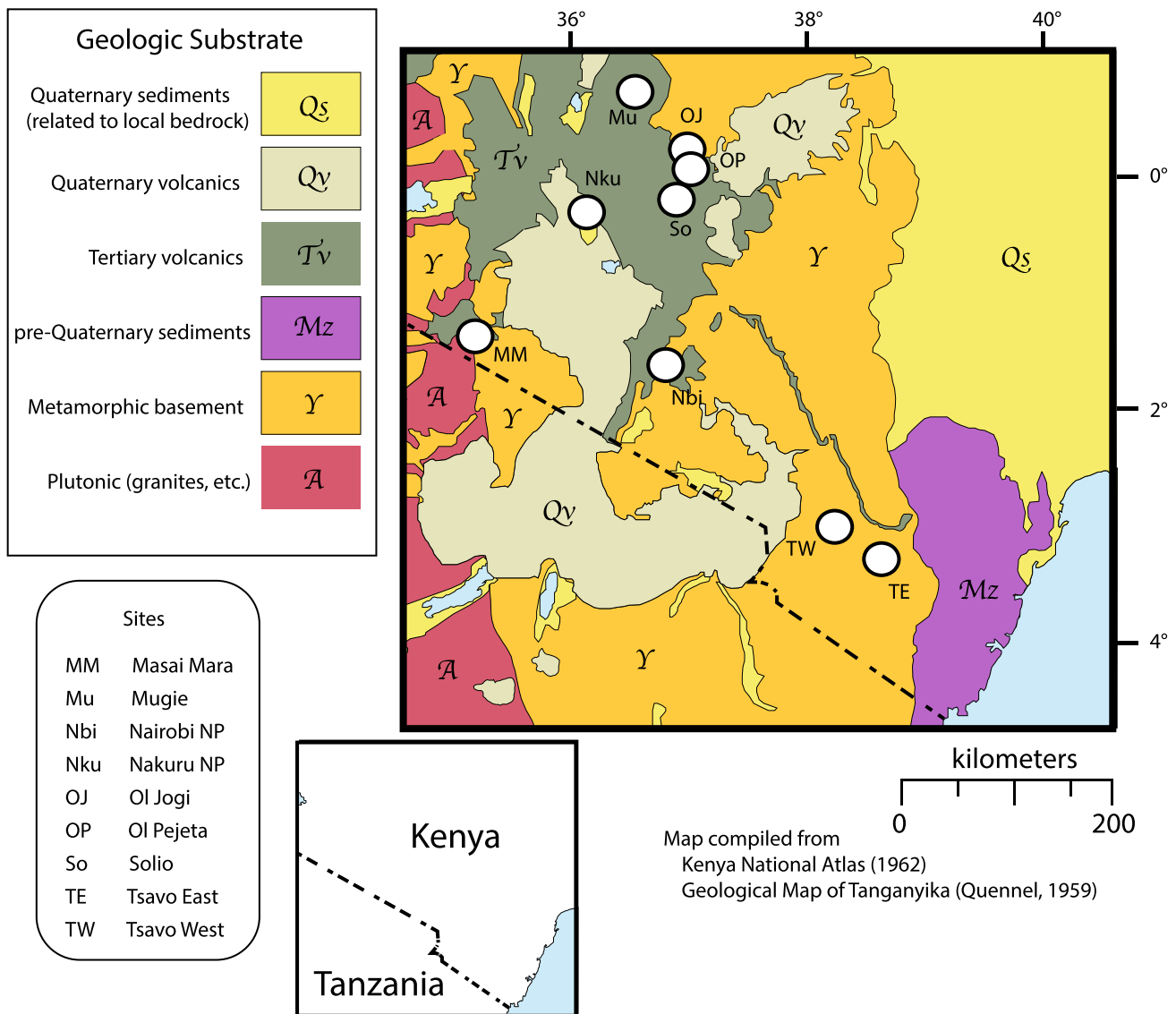
Fig. 1 Localities of samples collected for isotope analysis in relationship to bedrock geology. Geology is from the Kenya National Atlas (1962) and the Quennel (1959). This figure will appear in color in the online version of the journal

Light stable isotope analysis

Single bulk hair samples and plant samples were ground and ca. 0.5–2 mg were analyzed using an elemental analyzer (EA) coupled to a flow-through isotope ratio mass spectrometer (IRMS) for measurements of the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios. Tooth enamel was treated with H_2O_2 and 0.1 m acetic acid and the dried powder was reacted with 100% H_3PO_4; purified CO_2 was analyzed for $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios on an IRMS operating in dual-inlet mode.

Results are reported using the conventional permil (‰) notation:

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000,$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios in the sample and standard, respectively. Analogous results are reported for $\delta^{15}N$ and $\delta^{18}O$ isotope values. The standards are Pee Dee Belemnite (PDB) for carbon and oxygen and AIR (atmospheric nitrogen) for nitrogen. We do not correct for the $\delta^{13}C$ change in the atmosphere because the change in $\delta^{13}C$ over the observation period (1997–2012) is less than 0.5 ‰ for the average annual atmosphere $\delta^{13}C$ value (NOAA 2017). We also do not correct the $\delta^{13}C$ values for the rhino tooth enamel samples, which were predominantly from about 1970; the rhino tooth enamel samples are directly compared with other taxa from the sample collection period and so all taxa would have an identical offset.

Sequential hair samples were cut into 2 mm segments and analyzed using the EA-IRMS as described above. Diet histories were modeled using the reaction progress model (Ayliffe et al. 2004; Cerling et al. 2007a); we use the same parameters as was found for equids (3 ‰ isotope enrichment for diet–hair for both $\delta^{13}C$ and $\delta^{15}N$; multi-pool model with 0.5, 4, and 138 days half-lives with fractional contributions of 0.41, 0.15, and 0.44, respectively). Zazzo et al. (2007, 2008) found that bovids (sheep and cow) also should be modeled with a multi-pool model with similar pool half-lives (i.e., one very short, one intermediate, and one ca. 100 days or longer). Turnover experiments of this nature have not defined the isotope pools for nitrogen with the same precision as carbon. Therefore, we use the same parameters for isotope turnover for nitrogen isotopes as for carbon isotopes; we base this rationale on the assumption that the turnover pools relate to essential versus non-essential amino acids and that the structural remodeling of the different amino acids is similar for carbon and nitrogen. We assume a constant growth rate for rhino tail hair: West et al. (2004) and Wittemyer et al. (2009) have shown that changes in growth rates in horse tail hair related to diet, and in elephant tail hair related to physiological stress, respectively, must be less than 5%, which was the limit of detection in those two studies. Average growth rates are not known for rhinoceros tail hair; we assume a growth rate of 0.8 mm per day, which is the same as that for equids and elephants (Ayliffe et al. 2004; West et al. 2004; Cerling et al. 2009; Wittemyer et al. 2009).

Sr isotope analysis

Sr isotope measurements on bulk rhino hair were made in the Sr isotope laboratory on a Neptune multi-collector ICP-MS in the Department of Geology and Geophysics at the University of Utah. Samples were digested and analyzed as described by Tipple et al. (2013), using the Sr-FAST method of Mackey and Fernandez (2011).

Results

Bulk samples, resident individuals

Hair of several individuals from Nairobi National Park, Tsavo West National Park (Ngulia), Tsavo East National Park, Masai Mara National Reserve, Nakuru National Park, and from Solio, Ol Pejeta, Ol Jogi, and Mugie in the greater Laikipia region were sampled and analyzed for $\delta^{13}C$ and $\delta^{15}N$ in their hair. Hair samples were generally 50–100 mm in length; reported values represent the average for an entire hair. Figures 2 and 3 show the average values of resident individuals from these regions which span a large ecological gradient from mesic (Nakuru, Nairobi, Masai Mara) to xeric
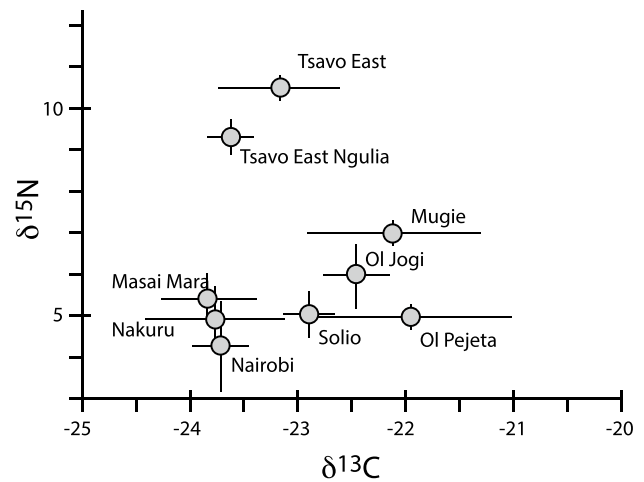

Fig. 2 Average $\delta^{13}C$ and $\delta^{15}N$ for rhino hair collected from different localities in Kenya

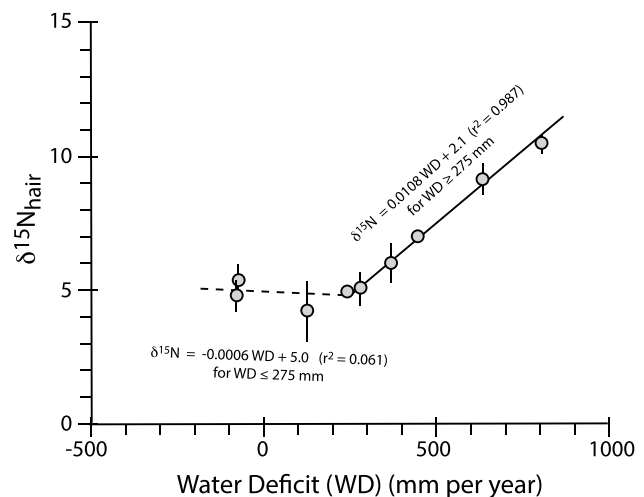**Fig. 3** Water deficit (WD) and average $\delta^{15}N$ for rhino hair from different localities. Below at WD of 275 mm/year there is no trend for WD vs. $\delta^{15}N$; however, above a WD of 275 mm/year there is a strong positive correlation ($r^2 = > 0.98$)

(Tsavo West); those results show that $\delta^{13}C$ and $\delta^{15}N$ values for many of the collecting regions differ from each other. Table 2 gives the average $\delta^{13}C$, $\delta^{15}N$, and $^{87}Sr/^{86}Sr$ isotope values for each locality and Table SI 1 gives the individual values for each analysis. The differences between the regions are discussed further in "Discussion".

Sequence samples of hair

Seven tail hairs were analyzed sequentially, one each from Tsavo East, Tsavo West, Nakuru, Mugi, Mara Mara, Ol Jogi, and Nairobi (Appendix SI 2). Figure 4 shows the $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ values for the hair along with the

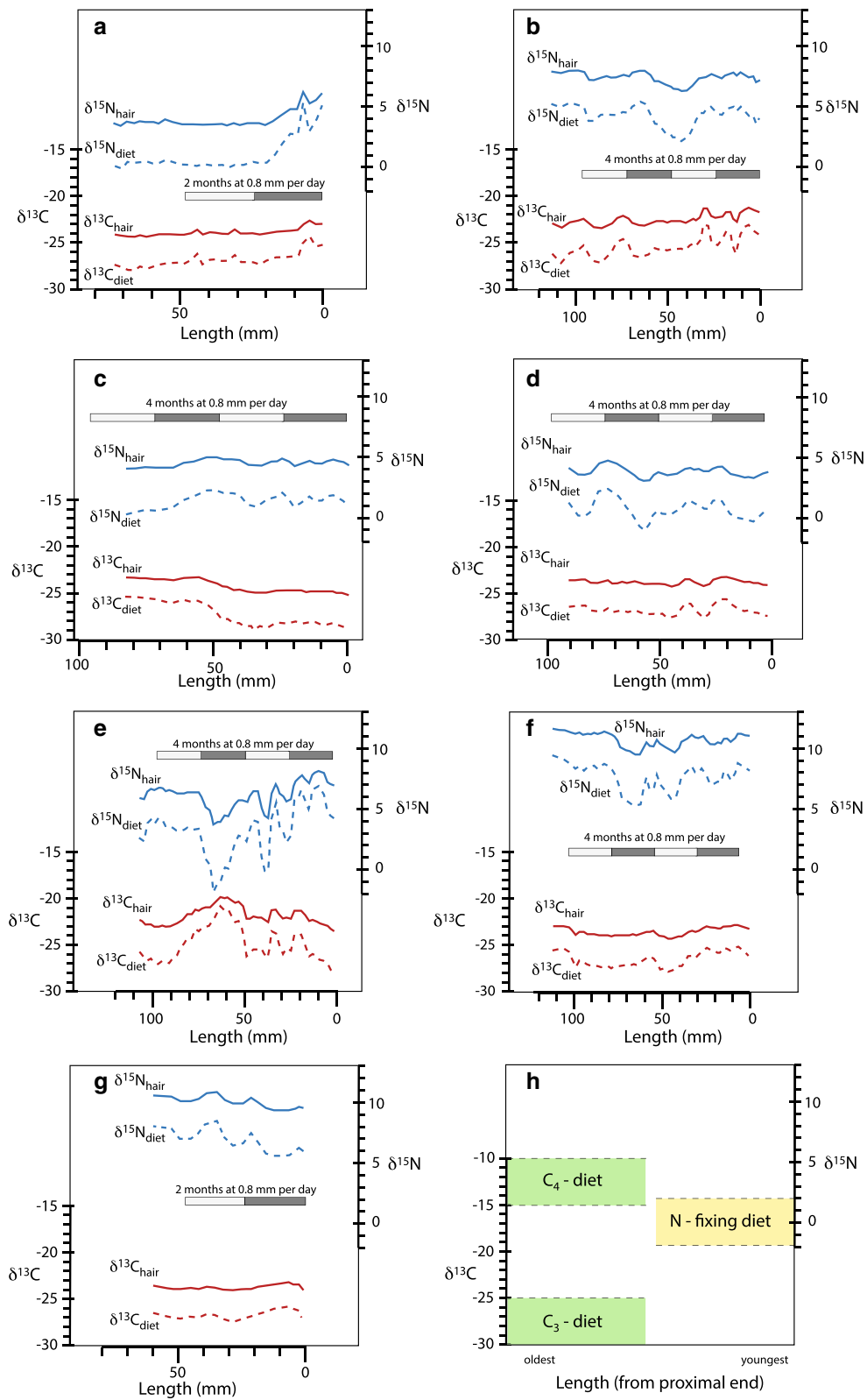**Fig. 4** $\delta^{13}C$ and $\delta^{15}N$ values for hair and estimated diets for sequential hair analysis of black rhinos from Kenya. **a** Masai Mara; **b** Mugie; **c** Nakuru; **d** Nairobi; **e** Ol Jogi; **f** Tsavo East; **g** Tsavo West; **h** $\delta^{13}C$ and $\delta^{15}N$ ranges for diet. This figure will appear in color in the online version of the journal

estimated diet for each segment using the reaction progress model for diet–hair enrichment (Ayliffe et al. 2004; Cerling et al. 2007a). Only one sample, from Ol Jogi in the Laikipia, shows evidence for a significant (> 20%) C_4/CAM contribution to the diet.

Plant samples

Table SI 2 summarizes the results from plants collected in the Laikipia, Nairobi, and Tsavo regions from 1997 to 2007 and Appendix SI 3 lists the individual results of analyses. As is typical for C_3 plants, the average $\delta^{13}C$ for the different regions ranged between − 25.5 and − 28.6 ‰ with the most mesic region (Nairobi) having the most negative $\delta^{13}C$ values. Likewise as is typical for C_4 plants, the average $\delta^{13}C$ values for C_4 plants range from − 11.6 to − 12.9 ‰ for this period of time (1997–2007). Average $\delta^{15}N$ values for C_3 and C_4 plants at each site are similar: Nairobi (2.4 and 1.8 ‰, respectively), Laikipia (6.3 and 4.8 ‰, respectively), and Tsavo (9.8 and 10.8 ‰, respectively); these $\delta^{15}N$ values represent a strong gradient from mesic (Nairobi) to xeric (Tsavo) as shown by the climatological data in Table 1. All sites had some samples that can be interpreted as being N-fixing based on $\delta^{15}N$ values near 0 ‰; however, most *Acacia* specimens (often considered to be an N-fixer) had $\delta^{15}N$ values that do not provide evidence for N-fixation by the plants sampled.

CAM plants were not analyzed as part of this study. In the discussion below we assume that CAM plants have a $\delta^{13}C$ value similar to C_4 plants growing in the same region. We discuss diet in terms of C_3 and C_4/CAM contributions as mentioned in "Methods".

Tooth enamel samples

Table SI 3 shows the results for analysis of tooth enamel for 14 different teeth from 10 individual rhinos, 11 elephants, 5 zebras, and 5 giraffes, all from Tsavo East National Park. All specimens were from the same approximate year of death (ca. 1970). The average $\delta^{13}C_{enamel}$ values are − 10.6 ± 1.3, − 9.9 ± 1.3, 0.4 ± 0.3, and − 11.3 ± 0.5 ‰, for rhinos, elephants, zebra, and giraffes, respectively.

Discussion

Hair samples used for comparison report the average of $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ over the length of the hair, which was generally 5 cm or greater. Assuming growth rates of 0.8 mm per day, the $\delta^{13}C$ and $\delta^{15}N$ values give the average value over several months of time, assuming 50% contribution to a longer metabolic pool increases the time represented in a sample.

End-member $\delta^{13}C$ values for diet estimates

The selection of an end-member diet value is problematic in many studies. In this case, we have seven detailed diet histories from different regions that can take into account seasonality in diet. We use the forward diet model of Ayliffe et al. (2004) and Cerling et al. (2007a) on seven individuals to estimate the "pure C_3" end member. Assuming growth rates of 0.8 mm per day, a sample interval of 2 mm gives an average length of 2.5 days. The 10th percentile diet values for all sites are between − 26.5 and − 28.6 ‰; due to the variability in individual plants (Appendix SI 3) we use − 27.5 ‰ for the C_3 end member. The pure C_4/CAM end member is assumed to be − 12.0 ‰ based on the data in Appendix SI 3, and the Cerling and Harris (1999) survey

Table 1 Location of site with rhino hair collections (2008–2012)

Location	Lat (N)	Long (E)	Altitude (m)	MAT (mm/year)	MAP (mm/year)	Water deficit (mm/year)
Masai Mara	− 1.41	35.08	1510	16.5	736	− 72
Mugie	0.74	36.63	1840	16.7	660	450
Nairobi NP	− 1.36	36.85	1643	18.9	785	124
Nakuru NP	− 0.46	36.09	1790	17.5	909	− 82
Ol Jogi	0.24	36.98	1720	17.4	770	372
Ol Pejeta	0.04	36.93	1790	16.2	819	260
Solio	− 0.24	36.88	1995	16.0	758	275
Tsavo East	− 3.36	38.61	530	25.1	549	799
Tsavo West–Ngulia	− 3.05	38.30	585	25.0	621	629

Mugie and Ol Jogi MAP from Georgiadis (personal communication), Solio from Lamuria (Kenya Met Office), Tsavo West from Makindu

WD calculated as in Blumenthal et al. (2017)

of Kenyan C_3 and C_4 plants. For hair samples using a 3 ‰ isotope enrichment for ^{13}C (Cerling and Harris 1999), these correspond to $\delta^{13}C$ hair values of -24.6 and -9.0 ‰ for pure C_3 and pure C_4 diets, respectively.

Bulk hair samples: stable isotopes—$\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$

The $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ in hair from different regions in Kenya show clear differences in isotope values between Laikipia (Solio, Ol Pejeta, Ol Jogi, Mugie), Ngulia/Tsavo, Mara, Nakuru and Nairobi (Fig. 2 and Table 2). Mara, Nakuru, and Nairobi are depleted in both ^{13}C and ^{15}N; using the $\delta^{13}C$ values above the long-term fraction of C_4/CAM derived resources are ca. 5–10%. The Laikipia group (Solio, Ol Pejeta, Ol Jogi, Mugie) cluster together with $\delta^{13}C$ values indicating a C_4/CAM diet contribution of ca. 15–20% and with $\delta^{15}N$ values lower than the greater Tsavo region. The two Tsavo populations (Ngulia, and Tsavo East) have a low contribution of C_4/CAM (less than 10%), but have the highest $\delta^{15}N$ values.

Black rhinos are known to eat C_4 grasses, but some CAM plants are also likely to be of significant importance—specifically *Sansevieria, Aloe, Euphorbia,* and *Salsola,* all of which are known to be favored by black rhinos in certain regions or seasons (Goddard 1970; Oloo et al. 1994; Ganqa et al. 2005; Lieverloo et al. 2009; Buk and Knight 2010).

$\delta^{15}N$ is strongly related to the water deficit; Fig. 3 shows that there is little relationship between water deficit and $\delta^{15}N$ in rhino hair below ca. 275 mm/year, but that there is a very strong relationship above 275 mm/year ($r^2 > 0.98$). The $\delta^{15}N$ of plants from the Nairobi, Laikipia, and Tsavo regions show similar increases in $\delta^{15}N$ with increasing water deficit; the advantage of using $\delta^{15}N$ of hair as a proxy for the $\delta^{15}N$ of plants is that it averages the $\delta^{15}N$ values over many thousands of plants.

Strontium isotopes

We analyzed 51 bulk rhino hair samples and the $^{87}Sr/^{86}Sr$ ratio in the hair ranges from 0.7050 to 0.7136 (Table SI 2).

Comparison of the different sites shows that each site has a narrow range of $^{87}Sr/^{86}Sr$ ratios (Fig. 5). Sites in predominantly volcanic terrains (Fig. 1: Nakuru NP, Nairobi NP, and the Laikipia region) have $^{87}Sr/^{86}Sr$ values averaging between 0.706 and 0.707 (Table 2). Tsavo East and the Masai Mara regions, which have bedrock dominated by old Mozambique Belt metamorphic rocks (Fig. 1), have distinctive radiogenic $^{87}Sr/^{86}Sr$ ratios averaging about 0.710–0.711 (Table 2). The rhinos from Tsavo West are in the enclosed Ngulia Rhino Sanctuary; rhinos from this site may be on a mixing line between the Mozambique Belt end-member values (ca. 0.711) and a Quaternary volcanic end member because the
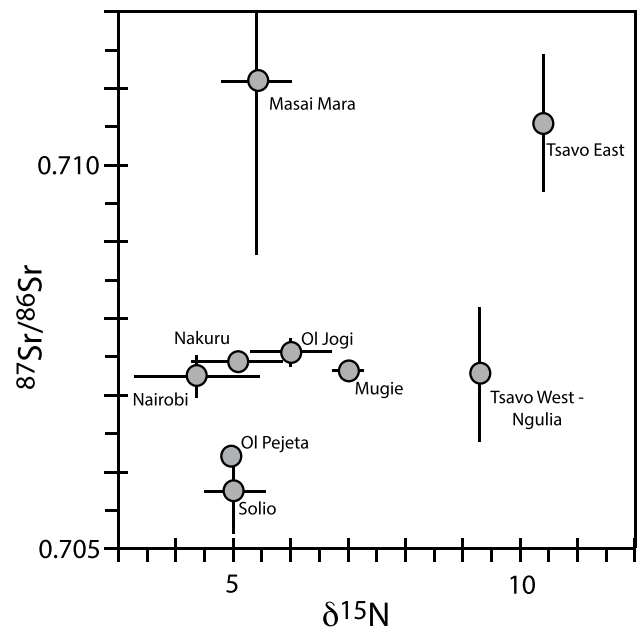

Fig. 5 Average $\delta^{15}N$ and $^{87}Sr/^{86}Sr$ for rhino hair collected from different localities in Kenya

Table 2 $\delta^{13}C$, $\delta^{15}N$, and $^{87}Sr/^{86}Sr$ isotope average values for rhino hair from localities in this study

Locality	$\delta^{13}C \pm 1sd$ (N)	$\delta^{15}N \pm 1sd$ (N)	$^{87}Sr/86Sr \pm 1sd$ (N)
Lake Nakuru NP	-23.8 ± 0.7 (13)	4.9 ± 0.8 (13)	0.7074 ± 0.0001 (7)
Masai Mara NR	-23.8 ± 0.5 (11)	5.4 ± 0.6 (11)	0.7111 ± 0.0023 (5)
Mugie GR	-22.1 ± 0.8 (15)	7.0 ± 0.3 (15)	0.7073 ± 0.0001 (10)
Nairobi NP	-23.7 ± 0.3 (16)	4.3 ± 1.1 (16)	0.7072 ± 0.0003 (5)
Ol Jogi GR	-22.5 ± 0.3 (9)	6.0 ± 0.8 (9)	0.7076 ± 0.0002 (5)
Ol Pejeta	-22.0 ± 0.9 (3)	5.0 ± 0.2 (3)	0.7062 ± 0.0002 (3)
Solio GR	-22.9 ± 0.3 (5)	5.0 ± 0.6 (5)	0.7057 ± 0.0005 (5)
Tsavo–Ngulia	-23.6 ± 0.2 (10)	9.3 ± 0.4 (10)	0.7073 ± 0.0009 (5)
Tsavo East NP	-23.2 ± 0.6 (6)	10.5 ± 0.3 (6)	0.7105 ± 0.0009 (6)

The number of samples analyzed in each group is given in parentheses

$^{87}Sr/^{86}Sr$ values for Mzima Springs is 0.70480 ± 0.00009 ($n = 3$). The Tsavo River originates in the Mzima Springs and is the principal drinking (and wallowing) water for rhinos in the Ngulia Rhino Sanctury, whereas the principal bedrock in the sanctuary is Mozambique Belt metamorphic rocks.

Possible forensics applications

These results suggest that stable isotopes could have forensic applications is helping to assign regions of origin to rhino horn. Because rhino horn is composed of keratin, the isotope distributions based on hair can give the $\delta^{13}C_{keratin}$, $\delta^{15}N_{keratin}$, and $^{87}Sr/^{86}Sr$ values characteristic of specific geographic regions (e.g., Figs. 2, 4). The current data set from East Africa for $^{87}Sr/^{86}Sr$ isotopes from wildlife from national parks and reserves in East Africa is restricted to only a few studies (van der Merwe et al. 1990; Koch et al. 1995; Coutu et al. 2016; and this study). Those studies suggest that the strontium isotope values in East Africa may range from ca. 0.704 (Quaternary volcanic rocks) to > 0.72 (Archean granitic rocks). This preliminary data set suggests that a more extensive sampling of wildlife from East Africa will be able to be used to show the ranges of $^{87}Sr/^{86}Sr$ isotope ratios related to geological bedrock maps. Thus, probability assignments to specific national parks/reserves or game ranches may be possible in the near future using the relationship of $^{87}Sr/^{86}Sr$ in animal tissues to the local bedrock.

Any application of this would have to take into account that rhino horn would record the location where the horn was growing, which may or may not be the same location as where a poaching incident occurred. Rhino horn grows at a rate of about 30–60 mm per year, with lower growth rates in older individuals; rhino horns are up to 500 mm in length, with the tips being worn off with age (Pienaar et al. 1991). Thus, a 500 mm horn could have as much as 15 years of growth recorded. Rhino horn grows at its base and thus the most recent keratin in the horn (at the base) would be from the location where the poaching occurred; a sample from the horn tip could be many years earlier and if the individual had been translocated, such a sample would indicate the location where the keratin actually formed. Thus, a preferred sample would be from the newest possible growth of keratin.

Seasonal diet changes in single individuals

We analyzed a detailed diet history of seven individuals (Table SI 2). Figure 4a–g shows the measured $\delta^{13}C_{keratin}$ and $\delta^{15}N_{keratin}$ in hair and the calculated isotope value for instantaneous diet for the previous 50+ days for these individuals in Masai Mara (4A), Mugie (4B), Nakuru (4C), Nairobi (4D), Ol Jogi (4E), Tsavo East (4F), and Tsavo East–Ngulia (4G). Hairs were subsampled at 2–3 mm resolution, which

corresponds to about 2–4 days using the estimated growth rate of 0.8 mm per day. We note that the growth is likely to be between 0.3 (humans) and 0.8 (elephants, equids) mm/day (West et al. 2004; Wittemyer et al. 2009); thus, estimated times in Fig. 4a–g should be construed as estimates only. Likewise, each sample interval represents the integrated diet in $\delta^{13}C$ and $\delta^{15}N$ over the ca. 2–4 days period represented in the interval analyzed; therefore, the range of true dietary $\delta^{13}C$ and $\delta^{15}N$ values from individual plants that contribute to diet over each hair is greater than the integrated dietary estimates that are shown in Fig. 4a–g.

The Mara rhino had a constant diet that changed about 2 weeks before the sample was collected (Fig. 4a). For the ca. 50 days prior to the late diet change, this individual had a $\delta^{13}C$ value of diet ranging between − 26.5 and − 28.4 ‰, consistent with a pure C_3 diet, or one with a small fraction (< 5%) of C_4/CAM. However, in the last 2 weeks before sampling, the diet was a high as − 24.6 ‰, which we estimate indicates up to 20% C_4/CAM. Nitrogen isotope values also are different in the final 2 weeks before sampling: the first 2 months diet was between 0 and 1 ‰ for $\delta^{15}N$, whereas the last several weeks (the period where C_4/CAM became significant) was as high as + 5 ‰. We do not have representative samples of plants from the Mara and so do not speculate on the $\delta^{15}N$ of plants in the region, except to note that mesic environments in Kenya (e.g., Nairobi) have more positive $\delta^{15}N$ values than the more xeric regions (Laikipia, Tsavo) as shown in Appendix SI 3.

The Mugi rhino hair was 116 mm long and represents a time interval of about 5 months (150 days) based on the estimated growth rates of 0.8 mm per day; Fig. 4b shows the $\delta^{13}C$ and $\delta^{15}N$ of hair and also the estimated $\delta^{13}C$ and $\delta^{15}N$ of their diet throughout this period. Estimated diet $\delta^{13}C$ values of range from − 23.0 to − 27.5 ‰, which is in the range of the $\delta^{13}C$ value for C_3 plants from water-stressed regions. Using a $\delta^{13}C$ value of − 27.5 ‰ for C_3 plants, one could make the case for a diet that includes up to ca. 20% C_4/CAM-derived vegetation. This sample was collected in January 2012, after the inception of the "short rains", and the highest $\delta^{13}C$ values were during the period when grasses would have been at their highest level of nutrition. Thus, this individual likely had a small, but significant, fraction of C_4/CAM vegetation in its diet for a short period of time. $\delta^{15}N$ values of the estimated diet range from 2.3 to 5.5 ‰; the $\delta^{15}N$ values of *Acacia* in the Laikipia region have a very wide range of values, and their $\delta^{15}N$ values indicate that most *Acacia* in Laikipia are not N-fixing (see Table SI 3)

The Nakuru rhino hair was 85 mm long, representing a time interval of ca. 110 days based on an assumed growth rate of 0.8 mm per day. This individual exhibited little variation in the $\delta^{13}C$ and $\delta^{15}N$ of the hair, and hence little variation in the $\delta^{13}C$ and $\delta^{15}N$ of the diet over this time interval (Fig. 4c). The average $\delta^{13}C$ of the diet was calculated to be

$- 27.6 \pm 1.1$ ‰, indicating a nearly pure C_3 diet. The average $\delta^{15}N$ diet was estimated to be 1.6 ± 0.5‰.

The Nairobi rhino can be interpreted as having a pure C_3 diet throughout the recorded interval (Fig. 4d); diet ranged between $- 25.8$ and $- 27.8$ ‰ which is in the observed range of C_3 plants for Nairobi (Cerling and Harris 1999). The calculated $\delta^{15}N$ value for the diet ranged from 2.4 to $- 0.8$ ‰; many plants in the Nairobi region have values in this range (Appendix SI 3) including N-fixing plants as well as plants that are not N-fixing.

The Ol Jogi rhino (Fig. 4e) shows significant diet change though the period recorded in its hair, which is ca. 135 days based on an estimated growth rate of 0.8 mm per day. Weekly diet ranges from $- 28.1$ ‰ which is a pure-C_3 diet, to $- 21.4$ ‰ which is about 40% C_4/CAM. Approximately, 30% of the period of record has a diet comprising > 20% C_4/CAM (that is, more positive than $- 24$ ‰); about 40% of the time the diet was essentially all C_3 (more negative than ca. $- 26$ ‰). The estimated $\delta^{15}N$ of diet ranges from $- 1.5$ to 6.7 ‰, which is well within the range of $\delta^{15}N$ for plants in the Laikipia region. Most acacias in the Laikipia region have $\delta^{15}N$ values greater than 0 ‰, indicating that they do not directly fix nitrogen (Appendix SI 3).

The Tsavo East rhino hair was 115 mm in length, corresponding to a time interval of ca. 140 days based on the estimated growth rate of 0.8 mm per day and thus represents on the order of 5 months of time. The range in $\delta^{13}C$ of estimated diet is from $- 27.8$ to $- 25.0$ ‰ (Fig. 4f), indicating a pure C_3 diet or nearly so; the $\delta^{13}C$ of plants in Tsavo East ranges between $- 29$ and $- 24$ ‰ (Appendix SI 3). The $\delta^{15}N$ of the estimated diet range ranges from 5.4 to 9.6 ‰ which is well within the observed range of plants, including *Acacia*, within the Tsavo East region (Appendix SI 3). Overall, the diet of this rhino is suggestive of a pure C_3 diet, or nearly so, over the time interval represented by this individual.

The Tsavo West rhino was from the Ngulia sanctuary and the hair analyzed was 63 mm in length. The estimated $\delta^{13}C$ of the integrated diet ranged from $- 27.6$ to $- 25.7$ ‰ which represents a pure C_3 diet, or nearly so, over the ca. 3 months period represented by this sample (Fig. 4g). The range in integrated $\delta^{15}N$ values is slightly higher (ca. 3 ‰) over this interval.

Altogether, we analyzed the detailed seasonal diets, integrated over a ca. 2–4 days interval, over time periods of the order of 2–5 months per individual. Only one individual (from Ol Jogi) showed unequivocal evidence for some C_4/CAM diet resource use, although several others had $\delta^{13}C$ values high enough that they could indicate 10–20% C_4 diet intake, or that the local $\delta^{13}C$ was several per mil enriched due to water-stressed conditions. Overall, the detailed isotope profiles show relative constant diets over time, in significant contrast to elephants in East Africa (Cerling et al. 2004, 2006, 2009).

Rhino diet in Tsavo from the 1960s using tooth enamel

The diets of rhinos from Tsavo National Park in the 1960s were characterized using tooth enamel from 16 different individuals using the Tsavo East Research Centre collections (Table SI 3). Proportions of C_3-browse and C_4/CAM in the diet of rhinos and elephants were determined assuming that giraffe and zebra represent end-member values for the C_3-browse and the C_4/CAM diet, respectively. Using these end members gives estimates for black rhinos and elephants of 7 ± 11 and 13 ± 11% C_4/CAM in the diets, respectively, using the standard deviation from the mean. The range for C_4/CAM consumption was 0–23% for rhinos. The amplitude of diet change is attenuated during enamel formation and maturation (Passey and Cerling 2002) and, therefore, the seasonal input of C_4/CAM could be higher than 25%. Goddard (1970), reporting observations from 1967 to 1969, noted that rhinos in Tsavo Park consumed > 100 different species of plants from 32 plant families, that grass was rarely eaten, and that rhinos had a clear preference for legumes. These data show that several of the rhinos from the Tsavo National Parks had a small portion of C_4 grasses or other C_4–CAM plants contributing to their diet in Tsavo National Park in the 1960s; for comparison, the hair samples collected from 2008 to 2012 from the Tsavo National Parks suggest a pure C_3 diet for the rhinos sampled.

Conclusions

Stable isotope ratios from rhino hair from Kenya shows distinct differences between some geographic regions. All Nairobi rhinos have very similar $\delta^{13}C$ and $\delta^{15}N$ values; the $\delta^{13}C$ values are compatible with a diet that is nearly 100% from C_3 plants, and with $\delta^{15}N$ diet values near 0 ‰. This does not indicate a reliance on *Acacia* because many other plants have values similar to this in this mesic part of Kenya. Rhinos from Mara also have a diet comprising almost entirely C_3 plants, but most of them have a slightly higher $\delta^{15}N$ value than do Nairobi rhinos. Rhinos from Laikipia (Ol Jogi, Ol Pejeta, Mugie) show evidence for a portion of C_4/CAM in the diet; the percentage of C_4/CAM could reach quantities of up to 40% for short periods of time based on a detailed isotope profile in hair. Rhinos from Tsavo East have the highest $\delta^{15}N$ values; other mammals from the Tsavo region also have high $\delta^{15}N$ values (Cerling et al. 2004; Yeakel et al. 2009) and this is likely due to the xeric character of the landscape with associated high $\delta^{15}N$ values in many plants.

$^{87}Sr/^{86}Sr$ values of rhinos from our study areas range from about 0.705 to 0.714. Lower values, from 0.705 to 0.708, are associated with the regions where the local geology comprises primarily of Neogene and Quaternary volcanic rocks.

The highest values, from 0.709 to 0.714, are from Tsavo East and the Masai Mara, both of which are dominated by Mozambique Belt basement rocks. The ^{87}Sr/^{86}Sr ratios from Ngulia sanctuary rhino in Tsavo West appear to represent a mixing between the basement Mozambique Belt rocks and the waters derived from Mzima Springs that are sourced in young Quaternary volcanics. Rhinos from Nakuru and Nairobi have ^{87}Sr/^{86}Sr ratios between 0.7069 and 0.7075; Laikipia rhinos have ^{87}Sr/^{86}Sr values between 0.705 and 0.708, with increasing ^{87}Sr/^{86}Sr ratios from the southeast (Solio–Ol Pejeta) to the northwest (Ol Jogi–Mugie).

In summary, the light stable isotopes (δ^{13}C and δ^{15}N) are good indicators of local ecology, whereas ^{87}Sr/^{86}Sr ratios indicate local geological bedrock. Together, these suggest a possible application for forensic use as well as having ecological applications.

Author contribution statement TEC and SAA conceived and designed the experiments. TEC, SAA, FG, JMK, LK, JWK, CK, IL, and ANM carried out the field work and laboratory analyses were performed by ANM, CRA, DPF, LH, and SJT. TEC, SAA, and DPF analyzed the data. TEC and SAA wrote the manuscript.

References

Amin R, Okita-Ouma B, Adcock K, Emslie RH, Mulama M, Pearce-Kelly P (2006) An integrated management strategy for the conservation of Eastern black rhinoceros *Diceros bicornis michaeli* in Kenya. Int Zoo Yearb 40(1):118–129

Ayliffe LK, Cerling TE, Robinson T, West AG, Sponheimer M, Passey BH, Hammer J, Roeder B, Dearing MD, Ehleringer JR (2004) Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet. Oecologia 139:11–22

Birkett A (2002) The impact of giraffe, rhino and elephant on the habitat of a black rhino sanctuary in Kenya. Afr J Ecol 40:276–282

Blum JD, Taliaferro EH, Holmes RT (2001) Determining the sources of calcium for migratory songbirds using stable strontium isotopes. Oecologia 126:569–574

Blumenthal SA, Levin NE, Brown FH, Brugal J-P, Chritz KL, Harris JM, Jehle GE, Cerling TE (2017) Aridity and hominin environments. Proc Natl Acad Sci 114:7331–7336

Brennan SR, Fernandez DP, Zimmerman CE, Cerling TE, Brown RJ, Wooller MJ (2015a) Strontium isotopes in otoliths of a non-migratory fish (slimy sculpin): implications for provenance studies. Geochim Cosmochim Acta 149:32–45

Brennan SR, Zimmerman CE, Fernandez DP, Cerling TE, McPhee MV, Wooller MJ (2015b) Strontium isotopes delineate fine-scale natal origins and migration histories of Pacific salmon. Sci Adv 1(4):e1400124. https://doi.org/10.1126/sciadv.1400124

Buk KG, Knight MH (2010) Seasonal diet preferences of black rhinoceros in three arid South African National Parks. Afr J Ecol 48(4):1064–1075

Cerling TE, Harris JM (1999) Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. Oecologia 120:347–363

Cerling TE, Harris JM, Passey BH (2003) Dietary preferences of East African Bovidae based on stable isotope analysis. J Mamm 84:456–471

Cerling TE, Passey BH, Ayliffe LK, Cook CS, Ehleringer JR, Harris JM, Dhidha MB, Kasiki SM (2004) Orphans' tales: seasonally dietary changes in elephants from Tsavo National Park, Kenya. Palaeogeogr Palaeoclimatol Palaeoecol 206:367–376

Cerling TE, Wittemyer G, Rasmussen HB, Vollrath F, Cerling CE, Robinson TJ, Douglas-Hamilton I (2006) Stable isotopes in elephant hair documents migration patterns and diet changes. Proc Natl Acad Sci 103:371–373

Cerling TE, Ayliffe LK, Dearing MD, Ehleringer JR, Passey BH, Podlesak DW, Torregrossa A-M, West AG (2007a) Determining biological tissue turnover using stable isotopes: the reaction progress variable. Oecologia 151:175–189

Cerling TE, Omondi P, Macharia AN (2007b) Diets of Kenyan elephants from stable isotopes and the origin of confiscated ivory in Kenya. J Afr Ecol 45:614–623

Cerling TE, Wittemyer G, Ehleringer JR, Remien CH, Douglas-Hamilton I (2009) History of Animals using Isotope Records (HAIR): a 6-year dietary history of one family of African elephants. Proc Natl Acad Sci 106:8093–8100

Coutu AN, Lee-Thorp J, Collins MJ, Lane PJ (2016) Mapping the elephants of the 19th century East African ivory trade with a multi-isotope approach. PLoS One 11(10):e0163606

Dharani N, Kinyamario JI, Wagacha PW, Rodrigues AJ (2009) Browsing impact of large herbivores on *Acacia xanthophloea* Benth in Lake Nakuru National Park, Kenya. Afr J Ecol 47:184–191

Emslie RH (2013) African rhinoceroses—latest trends in rhino numbers and poaching. CITES—CoP16 Inf. 51. An update to Doc 54-2-Annexe 2 from the IUCN Species Survival Commission's (IUCN/SSC) African Rhino Specialist Group to the CITES Secretariat pursuant to Resolution Conf. 9.14 (Rev. CoP15), pp 54-2

Ganqa NM, Scogings PF, Raats JG (2005) Diet selection and forage factors affecting woody plant selection by black rhinoceros in the Great Fish River Reserve, South Africa. S Afr J Wildl Res 35:77–83

Goddard J (1970) Food preferences of black rhinoceros in the Tsavo National Park. Afr J Ecol 8:145–161

Graustein WC, Armstrong RL (1983) The use of strontium-87/strontium-86 ratios to measure atmospheric transport into forested watersheds. Science 219:289–292

Hoppe KA, Koch PL, Carlson RW, Webb SD (1999) Tracking mammoths and mastodons: reconstruction of migratory behavior using strontium isotope ratios. Geology 27:439–442

Kennedy BP, Folt CL, Blum JD, Chamberlain CP (1997) Natural isotope markers in salmon. Nature 387:766–767

Kennedy BP, Blum JD, Folt CL, Nislow KH (2000) Using natural strontium isotopic signatures as fish markers: methodology and application. Can J Fish Aquat Sci 57:2280–2292

Kenya National Atlas (1962) Geological map of Kenya. Survey of Kenya. Nairobi, Kenya. 1 sheet

Koch PL, Heisinger J, Moss C, Carlson RW, Fogel ML, Behrensmeyer AK (1995) Isotopic tracking of change in diet and habitat use in African elephants. Science 267:1340–1343

Lieverloo RJ, Schulling BF, de Boer WF, Lent PC, de Jong CB, Brown D, Prins HHT (2009) A comparison of faecal analysis with backtracking to determine the diet composition and species preference of the black rhinoceros (*Diceros bicornis* minor). Eur J Wildl Res 55:505–515

Mackey GN, Fernandez DP (2011) American Geophysical Union Fall Meeting, San Francisco, CA, USA

Muya SM, Oguge NO (2000) Effects of browse availability and quality on black rhino (*Diceros bicornis michaeli* Groves 1967) diet in Nairobi National Park, Kenya. Afr J Ecol 38:62–71

Ngene S, Bitok E, Mukeka J, Okita-Ouma B, Gakuya F, Omondi P, Kimitei K, Watol Y, Kimani J (2011) Census and ear-notching of black rhinoceros (*Diceros bicornis michaeli*) in Tsavo East National Park, Kenya. Pachyderm 49:61–69

NOAA (2017) Carbon-13/carbon-12 ratios in carbon dioxide. https://www.esrl.noaa.gov/gmd/webdata/iadv/ccgg/graphs/pdfs/ccgg.MLO.co2c13.1.none.discrete.all.pdf. Downloaded on 11 March 2017

Okita-Ouma B, Mijele D, Amin R, Gakuya F, Ndeereh D, Lekolool I, Omondi P, Woodley D, Litoroh M, Bakari J, Kock R (2008) Minimizing competition by removing elephants from a degraded Ngulia rhino sanctuary, Kenya. Pachyderm 44:80–87

Okita-Ouma B, Amin R, Van Langevelde F, Leader-Williams N (2010) Density dependence and population dynamics of black rhinos (*Diceros bicornis michaeli*) in Kenya's rhino sanctuaries. Afr J Ecol 48:791–799

Oloo TW, Brett R, Young TP (1994) Seasonal variation in the feeding ecology of black rhinoceros (*Diceros bicornis* L.) in Laikipia, Kenya. Afr J Ecol 32:142–157

Passey BH, Cerling TE (2002) Tooth enamel mineralization in ungulates: implications for recovering a primary isotopic time-series. Geochim Cosmochim Acta 18:3225–3234

Patton F, Jones M (2007) Determining minimum population size and demographics of black rhinos in the Salient of Aberdare National Park, Kenya. Pachyderm 43:63–72

Patton F, Campbell P, Parfet E (2007) Establishing a monitoring system for black rhinos in the Solio Game Reserve, central Kenya. Pachyderm 43:87–95

Patton F, Campbell P, Parfet E (2008) Biological management of the high density black rhino population in Solio Game Reserve, central Kenya. Pachyderm 44:72–79

Pienaar DJ, Hall-Martin AJ, Hitchins PM (1991) Horn growth rates of free-ranging white and black rhinoceros. Koedoe 34:97–105

Quade J, Chivas AR, McCulloch MT (1995) Strontium and carbon isotope tracers and the origins of soil carbonate in South Australia and Victoria. Palaeogeogr Palaeoclimatol Palaeoecol 113:103–117

Quennel AM (1959) Geological map of Tanganyika. Geological Survey Department. Dodoma, Tanganyika (1 sheet)

Spinage C (1994) Elephants. T. & A.D. Prosser, London

Sponheimer M, Lee-Thorp JA, DeRuitter DJ, Smith JM, van der Merwe NJ, Reed K, Grant CC, Ayliffe LK, Robinson TF, Heidelberger C, Marcus W (2003) Diets of the Southern African bovidae: stable isotope evidence. J Mamm 8:471–479

Tipple BJ, Chau T, Chesson LA, Fernandez DP, Ehleringer JR (2013) Isolation of strontium pools and isotope ratios in modern human hair. Anal Chim Acta 798:64–73

van der Merwe NJ, Lee-Thorp JA, Bell RHV (1988) Carbon isotopes as indicators of elephant diets and African environments. Afr J Ecol 26:163–172

van der Merwe NJ, Lee-Thorp JA, Thackeray JF, Hall-Martin A, Kruger FJ, Coetzee H, Bell RHV, Lindeque M (1990) Source area determination of elephant ivory by isotopic analysis. Nature 346:744–746

Vitousek PM, Kennedy MJ, Derry LA, Chadwick OA (1999) Weathering versus atmospheric sources of strontium in ecosystems on young volcanic soils. Oecologia 121:255–259

Vogel JC, Eglington B, Auret JM (1990) Isotope fingerprints in elephant bone and ivory. Nature 346:747–749

Walpole MJ (2002) Factors affecting black rhino monitoring in Masai Mara National Reserve, Kenya. Afr J Ecol 40:18–25

Walpole MJ, Morgan-Davies M, Milledge S, Bett P, Leader-Williams N (2001) Population dynamics and future conservation of a free-ranging black rhinoceros (*Diceros bicornis*) population in Kenya. Biol Conserv 99:237–243

West AG, Ayliffe LK, Cerling TE, Robinson TF, Karren B, Dearing MD, Ehleringer JR (2004) Short-term diet changes revealed using stable carbon isotopes in horse tail-hair. Funct Ecol 18:616–624

Western D (1982) Patterns of depletion in a Kenya rhino population and the conservation implications. Biol Conserv 24:147–156

Wittemyer G, Cerling TE, Douglas-Hamilton I (2009) Establishing chronologies from isotopic profiles in serially collected animal tissues: an example using tail hairs from African elephants. Chem Geol 267:3–11

Yeakel JD, Patterson BD, Fox-Dobbs K, Okumura MM, Cerling TE, Moore JW, Koch PL, Dominy NJ (2009) Cooperation and individuality among man-eating lions. Proc Natl Acad Sci 106:19040–19043

Zazzo A, Harrison SM, Bahar B, Moloney AP, Monahan FJ, Scrimgeour CM, Schmidt O (2007) Experimental determination of dietary carbon turnover in bovine hair and hoof. Can J Zool 85:1239–1248. https://doi.org/10.1139/Z07-110

Zazzo A, Mononey AP, Monahan FJ, Scrimgeour CM, Schmidt O (2008) Effect of age and food intake on dietary carbon turnover recorded in sheep wool. Rapid Commun Mass Spectrom 22:2937–2945