Stable isotope ecology of the common hippopotamus

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Abstract

The diet of African hippopotamids can be documented through stable carbon isotope ratios ($^{13}C/^{12}C$) analyses of enamel and other tissues. The common hippopotamus *Hippopotamus amphibius* is widely assumed to be a pure grazer; however, the $^{13}C/^{12}C$ ratios of modern *H. amphibius* show a higher fraction of dietary C_3 biomass than estimated from traditional observations. Isotope profiles of modern hair and modern tooth enamel confirm that *H. amphibius* has a variable diet in both the short- (seasonal) and long- (sub-decadal) time scales. Isotopic analyses of extant mammals from the same parks as the analyzed hippos provide comparative examples for diets of C_3-browsers and C_4-grazers. Oxygen isotope ratios ($^{18}O/^{16}O$) show that the hippo is consistently the most ^{18}O-depleted mammal in any one ecosystem; this directly reflects its semi-aquatic habitat.

Introduction

Members of the family Hippopotamidae are non-ruminant artiodactyls whose aquatic mode of life makes them unique in Africa among the large mammals. *Hippopotamus amphibius*, the common extant hippo, was formerly widespread in the lakes and rivers of sub-Saharan Africa and is frequently reported as a grazer. *Choeropsis liberiensis*, the endangered extant pygmy hippo, is restricted to West Africa where it is said to be more terrestrial and to exploit a mixture of browse and graze. *Hippopotamus* species are present in many fossil Pliocene and Pleistocene sites in Africa. In this paper we will refer to the common hippopotamus as the 'hippo' to be distinguished from the pygmy hippo.

Hippopotamus amphibius is an unmistakeable species, with a barrel-shaped body weighing up to 3000 kg (Kingdon, 1982). It is adapted to semi-aquatic habitat, and water is required for thermoregulation: therefore it is never found far from water. Hippos feed on terrestrial vegetation and many studies claim that their diet consists predominantly, or solely, of grasses (e.g. Kingdon, 1982; Eltringham, 1999). However, a recent study by Boisserie *et al.* (2005) challenges this assumption. Hippos have a chambered stomach and are referred to as 'pseudo-ruminants'; this strategy can effec-tively ferment grasses and other low quality foods (Eltringham, 1999).

Stable carbon isotopes can distinguish browsing and grazing behavior in East Africa where virtually all grasses utilize the C_4 photosynthetic pathway and browse plants (e.g. trees, shrubs, forbs) use the C_3 pathway. The two pathways have different $^{13}C/^{12}C$ ratios. Dietary differences are recorded in developing tissues of mammals with rela-tively constant isotopic fractionation (Cerling & Harris, 1999; Passey *et al.*, 2005b) and provide insight into an individual's diet.

Oxygen isotopes record information about the water balance and thermoregulatory strategies of mammals (Bocherens *et al.*, 1996). Hippos live in an aquatic habitat, and it is expected that their oxygen isotope values will record their strong reliance on water.

In this paper we examine the stable carbon and oxygen isotope composition of tooth enamel the common hippo from East Africa to determine diet habits on regional and lifetime scales. Hippos are generally considered to be pure (or nearly pure) grazers, yet some studies (e.g. Boisserie *et al.*, 2005) indicate that this is not necessarily so. We therefore test this by examining hippo diets from many environments across East Africa. We also examine the

variation within single individuals, both on short- and long-time scales. Furthermore, we examine diet shifts of hippo populations over the course of several decades within a single region. We compare the stable carbon and oxygen isotopes to other modern large East African mammals to compare hippo diets and water use to that of other large mammals.

Methods

We analyzed 310 modern hippo enamel and hair samples. Enamel samples comprised 92 different teeth from 75 individuals and six isotope profiles on canines (224 analyses). Canines grow continually and record most of the life history of an individual. A single hair from a hippo [Lulimbe, Democratic Republic of Congo (DR Congo)] was analyzed sequentially. We sampled modern vegetation during several surveys in Kenya and Uganda between 1999 and 2003, and modern waters between 1977 and 2003. We include results from other East African mammals analyzed in our laboratory.

Tooth enamel was prepared and analyzed following the standard procedures for the treatment of tooth enamel for stable isotope analysis (Lee-Thorp & van der Merwe, 1987; Koch, Tuross & Fogel, 1997). For stable isotope profiles on modern canines a single sample was taken along each *c.* 10 mm interval along the length of the canine. Isotope ratios are reported using the standard ‰ notation where

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

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. The standard is the isotope reference V-PDB for carbon and oxygen. All data were corrected to modern enamel samples reacted off-line at 25 °C.

Approximately 500 μg of hair or plant material was flash-combusted in an elemental analyzer–gas chromatography–isotope ratio mass spectrometer system for $\delta^{13}C$ and $\delta^{15}N$ with the results being reported relative the V-PDB standard for carbon and to AIR for nitrogen using the standard δ notation. δD and $\delta^{18}O$ in waters were measured using standard methods and are reported relative to the isotope standard V-SMOW. $\delta^{18}O$ on the V-PDB and V-SMOW scales are related by (Sharp, 2007):

$$\delta^{18}O_{\text{V-SMOW}} = 1.03091 \, \delta^{18}O_{\text{V-PDB}} + 30.91$$

Isotope enrichment ε^* is calculated as:

$$\varepsilon^* = \left(\frac{1000 + \delta_A}{1000 + \delta_B} - 1 \right) 1000$$

where δ_A and δ_B are on the same isotope reference scale. The asterisk '*' implies that the materials being compared are not at isotope equilibrium.

We modeled diet histories using and inversion model for tooth enamel profiles (Passey *et al.*, 2005a), and a forward model for hair profiles (Ayliffe *et al.*, 2004; Cerling *et al.*, 2007). Isotope turnover parameters are from Ayliffe *et al.*

(2004); parameters for enamel maturation are given in the appropriate tables or figure captions.

Results

Extant *H. amphibius*: $\delta^{13}C$ and $\delta^{18}O$

The average $\delta^{13}C$ and $\delta^{18}O$ for 92 different hippopotamus teeth from 75 different individuals was -3.5 ± 2.4‰ and -1.2 ± 2.0‰, respectively (see Supplementary Material Appendix S1 for data). Stable isotope profiles on canines from six different individuals included profiles where $\delta^{13}C$ and $\delta^{18}O$ were relatively constant along the length of the profiles and some with large ranges in $\delta^{13}C$ or $\delta^{18}O$ (profile data are summarized in Supplementary Material Appendix S2). A single 75 mm long hippo hair from the Lake Edward region (DR Congo) had an average $\delta^{13}C$ value of -14.2‰, with a range from -10.9‰ to -18.7‰.

Hippopotamus amphibius: $\delta^{13}C$ compared with grazing or browsing taxa

The average $\delta^{13}C$ value for 92 teeth from 75 different individuals analyses is -3.5 ± 2.4‰ (Table 1) and range from $+1.5$‰ to -13.7‰. Comparison with obligate grazers (alcelaphines, buffalo, waterbuck, warthog, zebra), browsers (elephants) and obligate browsers (giraffe, dikdik) shows that that mean $\delta^{13}C$ of hippos differs significantly from all these other mammals (ANOVA, Bonferroni's test, $P < 0.0001$ for hippos compared with listed taxa; Fig. 1, Table 2). Figure 2 is a histogram of $\delta^{13}C$ values for grazing taxa compared with hippopotamus and shows that the average $\delta^{13}C$ value for hippos is several ‰ more negative than the average value for obligate grazers.

Comparisons within one population

We analyzed 21 teeth from 21 individuals from one locality in Tanzania (Katavi National Park). The average $\delta^{13}C$ and $\delta^{18}O$ from this locality are -3.3 ± 1.7 and -2.9 ± 0.9, respectively. We randomly generated sub-sample sets between two and 20 samples to access sample size on the mean and standard deviation. The standard error of the mean decreased most significantly between three and five samples, indicating that $n = 4$ is sufficient to establish the mean value, a conclusion similar to that reached by Clementz & Koch (2001).

Comparison within one ecosystem at different times

We compared the ecosystem at three different times in the greater Lake Edward region (Uganda and DR Congo). This region is in the Albertine Rift and is a mesic savanna habitat with abundant C4 grasses (Lock, 1970). A 520 mm tusk collected in Queen Elizabeth Park in Uganda in *c.* 1970 gave a record of diet from about 1950 to 1970 (estimated growth rate of 25 mm year^{-1}: Passey *et al.*, 2005a). Analyses from molars from seven different individuals that died in 1997 or

Table 1 $\delta^{13}C$ values of obligate grazers and browsers and of hippos *Hippopotamus amphibus* and elephants *Loxodona africana* from Kenya

	$\delta^{13}C$	1 SD	1 SE	Med	Maximum	Minimum	n	Nom. C_4
Grazers								
Alcelaphins	1.9	1.2	0.1	2.1	3.9	−2.6	82	110
Buffalo	0.8	1.3	0.1	1.0	2.8	−4.3	83	102
Waterbuck	0.6	1.1	0.2	0.7	2.5	−1.8	30	100
Warthog	−0.5	1.2	0.2	−0.4	1.6	−4.0	41	92
Zebra	0.2	1.0	0.1	0.2	2.1	−3.0	86	97
Average grazer	0.6							
Browsers								
Giraffe	−12.8	1.5	0.3	−12.8	−10.1	−15.9	27	−1
Dikdik	−12.5	1.6	0.4	−12.4	−8.2	−14.8	21	1
Average browser	−12.7							
Hippo	−3.6	2.5	0.3	−2.8	1.5	−13.7	66	73
Elephant	−11.5	2.5	0.1	−11.7	−5.7	−16.3	280	8

Obligate grazers are the alcelaphines wildebeest *Connochaetes taurinus*, kongoni *Alcelaphus buselaphus*, topi/tiang *Damaliscus lunatus*; buffalo *Syncerus caffer*; waterbuck *Kobus ellipsiprymnus*; warthog *Phacochoerus africanus*; and zebra *Equus burchelli*. Browsers are giraffe *Giraffa camelopardalis* and dikdik (*Madoqua kirki* and *Madoqua guentheri*). Nominal C_4 fractions are taken from the average $\delta^{13}C$ value compared with the endmember values determined by the average $\delta^{13}C$ of the obligate grazers and the obligate browsers using a linear mixing model.
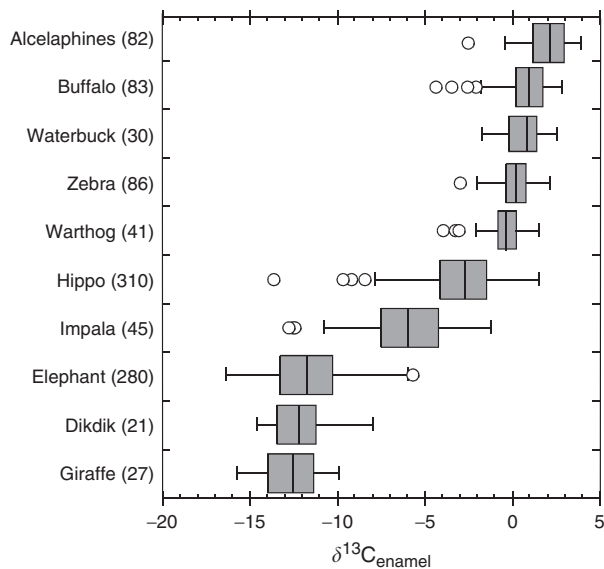

Figure 1 Box and whisker diagram showing $\delta^{13}C$ values for hippos and various grazers and browsers; open circles represent outliers. Data from Cerling *et al.* (2003) and unpublished data; number of analyses shown in parentheses for each species.

1998 in Queen Elizabeth Park give an estimate of diet between *c.* 1985 and 1995 (age estimates using Laws, 1968). These teeth were significantly different in $\delta^{13}C$ (ANOVA, Bonferroni's test, $P < 0.0001$) but not in $\delta^{18}O$ with respect to the 1970 sample (Fig. 3). Average $\delta^{13}C$ values were 0.0 ± 0.5‰ and 3.9 ± 1.8‰, and average $\delta^{18}O$ values were 0.0 ± 0.8‰ and 0.1 ± 0.6‰, respectively, for the older and younger populations. Carbon isotope values indicate that in the 1960s, hippo diet in Queen Elizabeth Park was comprised almost entirely of C_4 grass, but in the 1990s had a

significant C_3 component (*c.* 20–50%). The < 1‰ shift in the $\delta^{13}C$ of atmospheric CO_2 due to fossil fuel burning is much smaller than the change in $\delta^{13}C$ of hippo enamel (2–7‰) and contributes only a small fraction of the observed change in ^{13}C. Samples from the Lake Edward region in 2005 (Ishango and Lulimbe, Democratic Republic of Congo) have average $\delta^{13}C$ and $\delta^{18}O$ values of -2.9 ± 1.8‰ and -0.9 ± 1.2‰, respectively ($n = 15$). Neither the $\delta^{13}C$ nor the $\delta^{18}O$ differences between the mid-1990s and the mid-2000s were significant. Thus, in the period from *c.* 1960 to 2005, $\delta^{18}O$ did not change significantly. However, the $\delta^{13}C$ did change significantly: earlier diets of hippos in the Lake Edward region (pre-1970) were almost exclusively comprised of C_4 grass content whereas the later hippos (*c.* 1985–2005) had a diet comprised of 20–50% C_3 plants.

Comparison of hippopotamus to other large mammals within one ecosystem

We measured stable isotope analyses for large mammals from Tsavo National Park to compare with the hippopotamus (Table 3). $\delta^{13}C$ and $\delta^{18}O$ values are significantly different for the hippopotamus compared with all other species (ANOVA; Bonferroni's test, $P > 0.0001$ for all pairwise comparisons of hippopotamus with other species listed in Table 3).

Discussion

Diet of modern hippopotamus from East Africa

The ^{13}C in tooth enamel of grazers is enriched compared with that of hippos; grazers have average $\delta^{13}C$ values between −0.5‰ (suids) and +1.9‰ (alcelaphine bovids) whereas the hippo has an average $\delta^{13}C$ value of -3.6 ± 2.5‰

Table 2 *P*-values for $\delta^{13}C$ comparisons (Bonferroni) between different large taxa in East Africa

|	Alcelephin	Waterbuck	Buffalo	Zebra	Warthog	Hippo	Impala	Elephant	Dikdik	Giraffe
Alcelephin	1	0.04	0.01	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Waterbuck		1	>0.99	>0.99	>0.99	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Buffalo			1	>0.99	0.02	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Zebra				1	>0.99	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Warthog					1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Hippo						1	<0.0001	<0.0001	<0.0001	<0.0001
Impala							1	<0.0001	<0.0001	<0.0001
Elephant								1	>0.99	0.04
Dikdik									1	>0.99
Giraffe										1

Same dataset used as in Table 1.
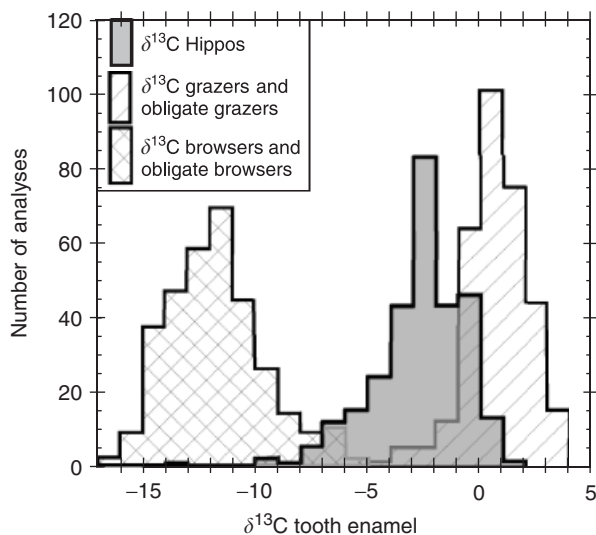
Figure 2 Histogram showing $\delta^{13}C$ values of hippos and obligate grazers (alcelaphine, waterbuck, buffalo, warthog, zebra). Data from Cerling et al. (2003) and unpublished data.

Figure 3 $\delta^{13}C$ and $\delta^{18}O$ values for hippo enamel collected in the Lake Edward region (Queen Elizabeth Park in1970 and 1998; from DR Congo in 2005). DR, Democratic Republic of Congo.

(92 teeth from 75 individuals). The isotopic enrichment factor for enamel compared with diet for large mammals is between 12‰ and 14‰ (Passey et al., 2005b); this gives an estimated $\delta^{13}C$ diet input between −17‰ and −15‰ for hippos. This leads to diet estimates between 60‰ and 75‰ C_4 biomass for the average hippopotamus diet in East Africa using mixing end member values (Cerling, Harris & Passey, 2003) for C_3 and C_4 plants for mesic (−27‰ and −12‰) and xeric environments (−26‰ and −13‰), respectively. The maximum $\delta^{13}C$ for hippos in this study was +1.5‰, similar to the maximum observed for warthogs (+1.6‰) from the same region for the same time interval. This is compatible with a pure C_4 biomass and therefore at least some hippos have a pure C_4 diet.

Stable isotope results show that, contrary to some interpretations based on observations (e.g. Field, 1970, 1972; Oliver & Laurie, 1974; Mackie, 1976; Grey & Harper, 2002; Codron et al., 2007) many hippos consume significant quantities of C_3 vegetation. However, several of these
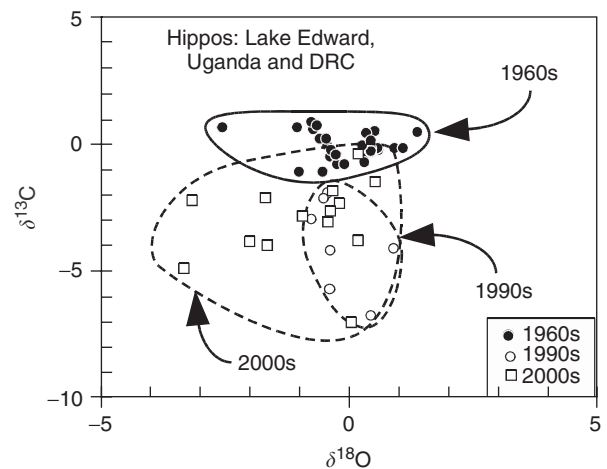
studies and others (e.g. Ansell, 1965; Mugangu & Hunter, 1992) report minor quantities of C_3 dicots in the diet. Our results concur with those of Boisserie et al. (2005) who concluded, from stable carbon isotopes, that hippos have a much higher C_3 component in their diet than is commonly believed. Few of the 75 individuals studied had a diet resembling that of a pure, or nearly pure, grazer.

Diet changes over time in single individuals

Stable isotope profiles were measured in canines from six hippos; an inversion model (Passey et al., 2005a) was used to estimate individual diet histories. Three profiles were from rogue individuals that invaded Arabuko-Sokoke National Park in about 1999 and were killed shortly thereafter; one was from an individual that died during a drought in Tsavo Park in January 1996, one was from Queen Elizabeth Park (d. c. 1970); and one was from the Laikipia region in Kenya (d. 2000). All except the Laikipia specimen were complete

Table 3 Comparison of δ^{13}C and δ^{18}O values of large mammals from a single region, Tsavo National Park, Kenya

Name	Species	*n*	δ^{13}C ($\pm 1\sigma$) (min., max.)	δ^{18}O ($\pm 1\sigma$) (min., max.)
Buffalo	*Syncerus caffer*	24	1.2 ± 1.0 (−2.5, 2.5)	1.7 ± 0.9 (0.5, 4.0)
Hippo	*Hippopotamus amphibius*	9	-3.6 ± 1.6 (−5.9, −1.7)	-2.7 ± 0.8 (−4.3, −1.6)
Lion	*Panthera leo*	9	-5.4 ± 1.4 (7.1, −2.7)	0.7 ± 0.5 (−0.2, 1.4)
Rhino	*Diceros bicornis*	22	-10.7 ± 1.2 (−12.9, −8.6)	0.7 ± 1.4 (−2.0, 3.1)
Zebra	*Equus burchelli*	13	0.3 ± 0.5 (−0.7, 1.0)	3.2 ± 1.6 (0.3, 6.0)
Giraffe	*Giraffa camelopardalis*	7	-11.9 ± 1.1 (−14.2, −10.7)	4.3 ± 0.8 (3.3, 5.5)
Waterbuck	*Kobus ellipsiprymnus*	12	1.0 ± 1.3 (−1.8, 2.5)	2.4 ± 0.9 (1.3, 4.5)
Elephant	*Loxodonta africana*	37	-9.7 ± 1.7 (−13.0, −5.7)	0.5 ± 0.6 (−0.9, 1.9)
Oryx	*Oryx beisa*	5	-0.7 ± 1.1 (−2.1, 0.4)	2.4 ± 1.7 (0.5, 5.1)

Maximum and minimum values shown in parentheses. Samples include both modern samples collected between 1997 and 2004, and archived material collected in the early 1970s (elephant and rhino only). The latter were not corrected for changes in the δ^{13}C of the atmosphere during that time (see Cerling & Harris, 1999). Specimens having more than one analysis are included as a single average value in this summary.
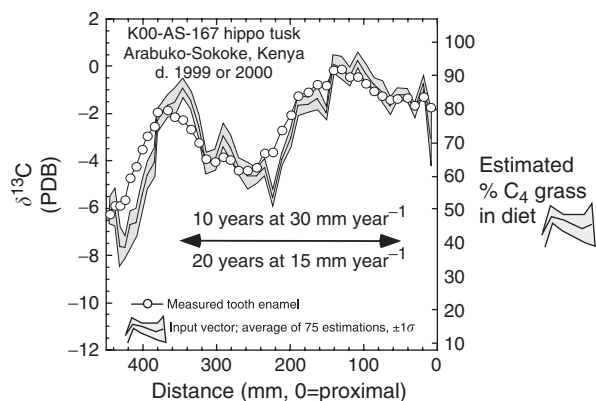

Figure 4 δ^{13}C profile canine from K00-AS-167 and estimate of δ^{13}C of body fluids using model of and the parameters described in Passey *et al.* (2005*a*). Circles indicate individual stable isotope values. Shaded areas represent the average ($\pm 1\sigma$) for 100 inversion runs for reconstructed δ^{13}C of initial tooth enamel for the time represented by each data point; this value is representative of enamel in equilibrium with the diet of that time step.

Figure 5 δ^{13}C and δ^{18}O profiles of canine from K01-TSV-291 (modeled as in Fig. 4). Circles indicate individual stable isotope values. Shaded areas represent the average ($\pm 1\sigma$) for 75 inversion runs for reconstructed δ^{13}C or δ^{18}O of initial tooth enamel for the time represented by each data point; these values are representative of enamel in equilibrium with the diet (δ^{13}C) or blood plasma (δ^{18}O) of that time step, respectively.

profiles from the proximal to distal ends; for the Laikipia specimen only the gum line to the distal end was sampled.

Two profiles had relatively constant δ^{13}C values: the Laikipia and Queen Elizabeth Park samples each had a δ^{13}C total range <2‰ (Supplementary Material Appendix S2); both individuals had diets that were predominantly C_4 grass.

The three profiles from hippos from the Arabuko-Sokoke region each had a large range in δ^{13}C, from 4‰ to 6‰ indicating a significant change in diet over their respective lifetimes; Fig. 4 shows the profile from one of these indivi-
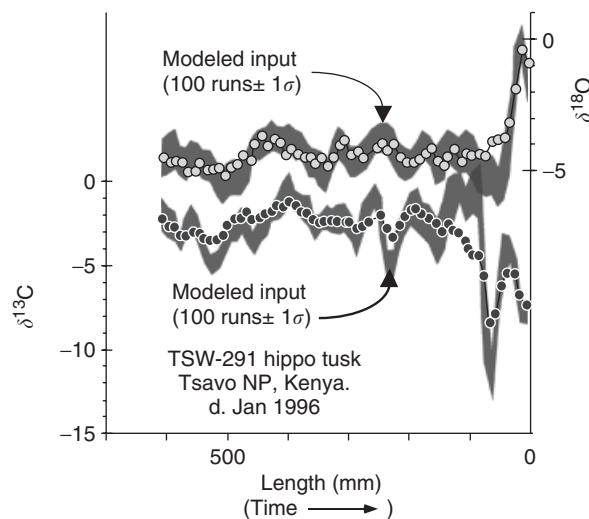
duals. Using growth rates between 15 and 30 mm year^{-1} this individual had periods of up to several years where the diet averaged *c.* 85% C_4, whereas for other long periods its diet was only *c.* 65% C_4.

The individual from the Tsavo region [K01-TSV-291 (KW/TW/HT/1/96)] had a consistently high fraction of C_4 biomass in its diet except for near the end of its life. Figure 5
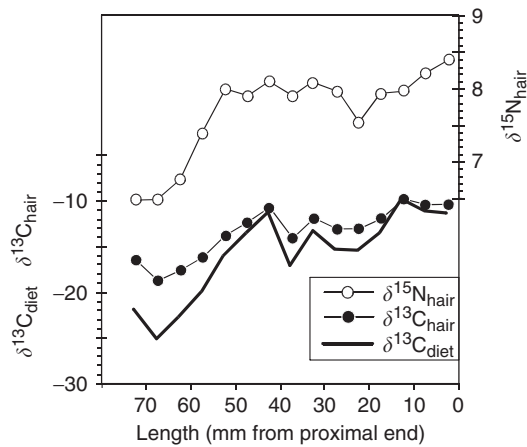
Figure 6 $\delta^{13}C$ profile of 75-mm-long hair from hippopotamus from Lulimbe, DR Congo, and estimated diet for each segment using model of Ayliffe et al. (2004) and Cerling et al. (2007).

shows the $\delta^{13}C$ profile derived from the mathematical inversion and indicates an abrupt diet change from a diet of $-1‰$ (c. 90% C_4) to $-12‰$ (c. 100% C_3) in just 20 mm of tusk growth (c. 1 year or less). $\delta^{18}O$ also undergoes a great shift shortly following this dietary crisis: an increase in 5.5‰ in 30 mm.

We analyzed a single hair from a hippo from Lulimbe in DR Congo killed by rebel soldiers during regional conflict. Lulimbe is on the southern shore of Lake Edward and is in a region with abundant C_4 grasses. A forward model (Ayliffe et al., 2004; Cerling et al., 2007) was used to estimate the dietary history. The growth rate of hippo tail hair is not known, but both equids and elephants have tail hair growth rates averaging c. 0.7 mm day^{-1} (Ayliffe et al., 2004; West et al., 2004; Cerling et al., 2006); we use this value to calculate the instantaneous diet of this individual. Figure 6 shows that while the $\delta^{13}C$ of hair varies from $-11‰$ to $-19‰$ over 75 mm, the diet required to produce this variation varies from about $-11‰$ to $-25‰$ indicating a diet varying from c. 100% C_4 to c. 100% C_3 biomass, respectively. This indicates a complete diet shift from almost exclusively C_4 grasses to almost exclusively C_3 plants over the period of a few months.

Diet change in hippos over time within a population

Further evidence of a significant C_3 component in hippo diet within a population through time is provided by samples from the Lake Edward region in the Albertine Rift. Average diet estimates are between c. 94‰ and 100‰ C_4 biomass before 1970 and between c. 55% and 75% C_4 biomass after 1980. A major cull of hippos occurred in Queen Elizabeth National Park in the early 1960s because of overpopulation of hippos and elephants. Analyses of stomach contents by Field (1970) showed that these hippos had a diet comprised of predominantly C_4 grasses; the isotope profile from the c. 1970 tusk is in agreement with that observation.

The differences in the carbon ratios between the two time periods may reflect the significant differences in large mammal density and forage availability between the 1960s and the 1990s. During the 1950s to 1960s, hippo and elephant populations were very large; in comparison, in the 1990s the populations of both elephants and hippos were seriously depleted from poaching. Elephants are predominantly browsers and had greatly reduced the available browse in much of the Queen Elizabeth National Park in the 1950s and early 1960s (Spinage, 1994).

Summary of modern hippo diets

Carbon isotopes in bulk tooth enamel, isotope profiles in hippo canines, and sequential profiles in hippopotamus tail hair, all show that H. amphibius has a diet that ranges from nearly pure C_4 grass to having a significant C_3 biomass component. None of the regions studied here (with the possible exception of Arabuko-Sokoke) support C_3 grasses, so the C_3 biomass must be from C_3 sedges or from C_3 dicots. Most published lists of estimated diets of hippopotamus have few, if any, C_3 plants listed. These results indicate that an important aspect of hippopotamus ecology, that of their diet or physiology, remains poorly understood. This agrees with the previous observations of Boisserie et al. (2005b), also based on carbon isotopes, that hippos can have a much higher component of C_3 biomass in their diets than is commonly believed.

Regional comparison of $\delta^{18}O$ of hippos in East Africa

Mammals derive water from several sources, including drinking (e.g. from springs, rivers, lakes, water holes), plant water (e.g. stems and leaves) and metabolic water (by reaction with carbohydrates). The isotopic composition of the water cycle is well described: meteoric water in the form of rainfall serves as a starting point from which the isotopic composition of water further evolves by isotope enrichment in ^{18}O. Evaporation from leaf surfaces and from open water sources (such as lakes) enriches ^{18}O by 5–15‰. In addition, cellulose and carbohydrates are enriched in ^{18}O compared with the source water; metabolic water, derived by the oxidation of carbohydrates is related to the $\delta^{18}O$ of the fixed oxygen and atmospheric oxygen (c. $+22‰$, but see discussion in Kohn, 1996). Therefore, water sources in large mammals can be considered to have an ^{18}O-depleted source (meteoric water) and several ^{18}O-enriched sources: namely, leaf water (Barbour & Farquhar, 2000; Helliker & Ehleringer, 2002) and metabolic water. A complication occurs when the drinking source itself is evaporated, such as in a lake; in those cases the drinking water would also be enriched relative to local meteoric waters.

Table 4 shows the $\delta^{18}O$ values for hippos from several localities in East Africa along with local waters. The isotope enrichment between local meteoric waters and tooth enamel carbonate is about 32‰ for hippos living in rivers, bearing in mind that the ^{18}O that is fixed in tooth enamel has several

Table 4 Estimates of stable isotopic composition of local meteoric waters and lake waters from samples discussed in text

Region	Waters $\delta^{18}O \pm 1\sigma$ (*n*) SMOW	Hippo $\delta^{18}O \pm 1\sigma$ (*n*) PDB	SMOW	ε^*
Meteoric sources (rivers, streams, waterholes)				
Baringo region	-3.2 ± 1.2 (7)	-1.0 ± 1.4 (2)	29.9	33.2
Laikipia – Mpala	-3.3 ± 0.9 (4)	-1.6 ± 0.7 (5)	29.3	32.7
Nairobi – Athi Plains	-4.0 ± 1.1 (15)	-2.6 ± 0.6 (3)	28.2	32.4
Naivasha region	-3.1 ± 1.7 (4)	-3.0 (1)	27.8	30.9
Turkana/Omo River	-3.0 ± 1.2 (11)	-2.3 (1)	28.5	31.6
Tsavo	-4.0 ± 1.1 (12)	-2.5 ± 0.6 (8)	28.3	32.5
Coast[a]	-2.3 (1)	-2.7 ± 1.0 (8)	28.1	30.5
Lakes				
Lake Turkana	5.6 ± 0.6 (25)	2.8 ± 1.0 (4)	33.8	28.0
Lake Edward	1.9 (1)	-0.2 ± 0.6 (8)	30.7	28.7
Lake Baringo	6.4 ± 2.1 (3)	-1.0 ± 1.4 (2)	29.9	23.3
Lake Naivasha	4.0 ± 3.1 (7)	-3.0 (1)	27.8	23.7

Meteoric waters include springs, rivers, and groundwater; waters were collected between 1977 and 2003. The isotope enrichment is calculated having converted $\delta^{18}O$ on the PDB scale to the SMOW scale; the number of analyses for hippos represents the number of individuals sampled. ε^* values are calculated for Baringo and Naivasha hippos using both meteoric and lake water sources.

[a]Coastal sample is from Lamu Island and represents local recharge in the coastal region and is used for the Arabuko-Sokoke samples.

discrete sources (i.e. drinking water, leaf water, metabolic water).

Hippos from the Lake Turkana–Omo River system provide a useful example of the importance of the drinking water source. Lake Turkana is a closed basin and is enriched in ^{18}O compared with local meteoric water (*c.* -3‰) or the inflowing Omo River (*c.* -1‰); it has an average $\delta^{18}O$ value between $+5$‰ and $+6$‰. The principal food source for both lower Omo River hippos and Lake Turkana hippos should have similar $\delta^{18}O$ values because local meteoric waters are the principal source of water for non-aquatic plants. Four hippos from Lake Turkana have $\delta^{18}O$ values that average $+2.8$‰ compared with -2.3‰ for a single hippo from the Omo River. Thus, hippos from freshwater closed basin lakes (e.g. Lake Turkana, Lake Naivasha) are likely to be enriched in $\delta^{18}O$ compared with hippos dwelling in nearby rivers. The isotope enrichment for the Lake Turkana hippos is 28.7‰ relative to drinking water whereas the Omo River hippos have enrichments typical for river-dwelling hippos (31‰).

Hippos from Queen Elizabeth Park in Uganda are less easily interpreted. Lake Edward is a few permil enriched compared with the inflowing waters in the Kazinga Channel and hippos freely move between these water sources. However, the data from Queen Elizabeth Park can bracket the isotope enrichment between source waters and tooth enamel. Kazinga Channel water (-1.7‰) gives an isotope enrichment of 32‰, whereas using Lake Edward water ($+1.9$‰) we calculate an isotope enrichment of 29‰.

Two localites were puzzling in our analysis of East African hippos. Two hippos from Lake Baringo had $\delta^{18}O$ values of 0‰ and -2‰, and a single hippo from Lake Naivasha had a $\delta^{18}O$ value of -3‰. Both Lakes Baringo and Naivasha are freshwater closed basin lakes and are highly enriched in $\delta^{18}O$ compared with the inflowing waters.

The individual histories of these hippos are not known; although they probably lived for some considerable time in their respective lakes. However, their $\delta^{18}O$ values are more compatible with unevaporated source waters. Additional samples from individuals with known histories are needed to resolve this dilemma.

Isotope ecology of hippos compared with other large mammals

We compare the stable isotope ecology of hippos with other large mammals in Tsavo National Park, Kenya (mean annual temperature: 24.9 °C; mean annual precipitation: 550 mm). As expected, hippos are depleted in ^{18}O compared with other large mammals (Table 3; Fig. 7); Bocherens *et al.* (1996) also observed that hippos were depleted in ^{18}O relative to other large mammals in nearby Amboseli. Elephants, rhinos and lions are enriched in ^{18}O by 3–3.5‰ compared with hippos; all three require water daily and are considered to be obligate drinkers. At the other end of the spectrum are zebras and giraffes, which are enriched by 6–7‰ in ^{18}O compared with hippos. Waterbuck, buffalo and oryx are intermediate between the dry-land water-dependent mammals and the relatively water-independent giraffe.

Conclusions

The diets of modern and fossil hippos can be studied using carbon isotopes from tooth enamel, hair and other tissues. Modern hippos have a much higher component of C_3 biomass in their diets than is normally suggested; this is extremely important for interpreting the behavior of hippos. Also, their diets are far more varied on short time scales than was previously assumed. Sequential analysis of hair and
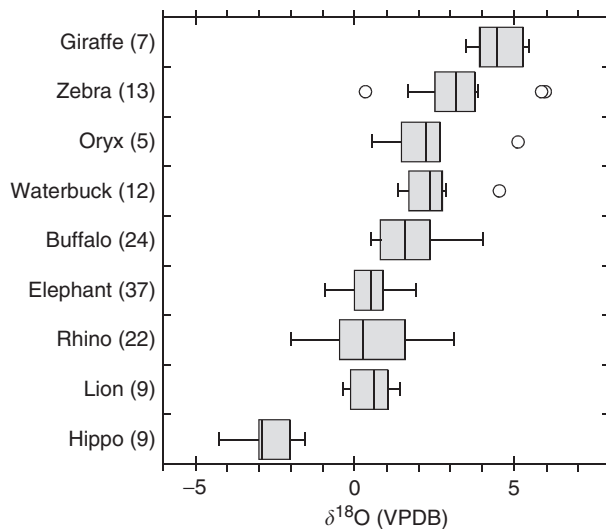
Figure 7 Box and whisker diagram of $\delta^{18}O$ for large mammals in the Tsavo National Park region, Kenya. Circles represent outliers. Number of individuals shown in parentheses; multiple samples from one individual were averaged and were treated as a single analysis.

canine tooth enamel reveals short-term and long-term changes in diets, respectively. In certain cases, the tissues record important events in the life of individuals.

Study of the oxygen isotope composition of tooth enamel shows that hippos are less enriched in ^{18}O than other mammals in the same ecosystem. This study confirms and expands on the observations of Bocherens *et al.* (1996). This study further suggests the possibility of setting up a hierarchy of $\delta^{18}O$ values to understand water utilization in fossil ecosystems (Levin *et al.*, 2006).

Acknowledgments

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Supplementary material

The following material is available for this article online:

Appendix S1. Locality and stable isotope data for modern hippopotamus (*Hippopotamus amphibius*) in this study (92 teeth from 75 individuals). Isotope profiles from canines presented as average values (summary data for profiles given in Appendix S2). Year is year collected (ca. 1–3 years of the year of death) or date of death (if known)

Appendix S2. Summary data for stable isotope profiles from modern hippopotamus (*Hippopotamus amphibius*) in this study

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Appendix 1. Locality and stable isotope data for modern hippopotamus (*Hippopotamus amphibius*) in this study (92 teeth from 75 individuals). Isotope profiles from canines presented as average values (summary data for profiles given in Appendix 2). Year is year collected (ca. 1-3 years of the year of death) or date of death (if known).

sample number	country	region	year	tooth	$\delta^{13}C$	$\delta^{18}O$
GNP-hippo	DR Congo	Garamba	1996	M	-5.8	-1.9
PNVN-009(average)	DR Congo	Ishango	2005	C-ave	-0.5	0.2
PNVN-009	DR Congo	Ishango	2005	M3	-3.9	-2.0
PNVIs-017	DR Congo	Ishango	2005	M2	-4.0	-1.7
PNVIs-021-m2	DR Congo	Ishango	2005	m2	-2.2	-3.2
PNVIs-021-m3	DR Congo	Ishango	2005	m3	0.1	-0.4
PNVIs-022-m3	DR Congo	Ishango	2005	m3	-3.1	-0.5
PNVIs-024-M2	DR Congo	Ishango	2005	M2	-7.1	0.0
PNVIs-024-P4	DR Congo	Ishango	2005	P4	-3.8	0.2
PNVL-004	DR Congo	Lulimbe	2005	M3	-2.2	-1.7
PNVL-008	DR Congo	Lulimbe	2005	p4	-2.8	-0.4
PNVL-011	DR Congo	Lulimbe	2005	M3	-1.5	0.5
PNVL-015	DR Congo	Lulimbe	2005	m2	-2.2	-0.2
PNVL-017	DR Congo	Lulimbe	2005	p4	-4.8	-3.4
PNVL-022	DR Congo	Lulimbe	2005	p3	-2.9	-1.0
PNVL-023	DR Congo	Lulimbe	2005	p3	-1.9	-0.3
Omo hippo	Ethiopia	Omo	2000	M	-5.1	-2.3
ET05-AWSH-27	Ethiopia	Awash NP	2005	M1	-7.0	-0.2
ET05-AWSH-28	Ethiopia	Awash NP	2005	m1	-4.1	0.9
ET05-AWSH-28	Ethiopia	Awash NP	2005	M1	-3.6	-0.5
ET05-AWSH-29	Ethiopia	Awash NP	2005	m2	-3.2	0.3
ET05-AWSH-29	Ethiopia	Awash NP	2005	M2	-2.9	0.7
ET05-NCHSR-01	Ethiopia	Nechisar NP	2005	m2	-2.4	3.3
ET05-NCHSR-02	Ethiopia	Nechisar NP	2005	M3	-1.8	3.4
ET05-NCHSR-03	Ethiopia	Nechisar NP	2005	m1	-4.1	3.3
ET05-NCHSR-05	Ethiopia	Nechisar NP	2005	m1	-7.9	2.3
ET05-NCHSR-06	Ethiopia	Nechisar NP	2005	m2	-5.0	2.3
ET05-NCHSR-07	Ethiopia	Nechisar NP	2005	m3	-4.0	1.6
K00-AS-165	Kenya	AS	2000	m2	-1.0	-1.9
K00-AS-166	Kenya	AS	2000	c-(ave)	0.0	-4.2
K00-AS-166-m2	Kenya	AS	2000	m2	-5.7	-1.5
K00-AS-167	Kenya	AS	2000	c-(ave)	0.0	-2.3
K00-AS-168 p2	Kenya	AS	2000	p2	-1.9	-1.7
K00-AS-168	Kenya	AS	2000	c-(ave)	0.0	-1.7
K00-AS-169 dP/4	Kenya	AS	2000	dP4	-6.4	-3.1
K00-AS-169 P/3	Kenya	AS	2000	p3	-4.0	-2.1
OM 2054	Kenya	Athi	1967	m1	-1.3	-1.9
OM 2054	Kenya	Athi	1967	m2	-2.9	-2.8
OM 6604	Kenya	Athi	1967	m1	-7.7	-2.9

NL 1	Kenya	Baringo	1992	dp4	-13.7	0.0
OM 2205	Kenya	Baringo	1968	m2	-9.7	-2.0
K01-202-LAI	Kenya	Laikipia	2000	p3	-2.7	-1.8
K01-LAI-202	Kenya	Laikipia	2000	M	-3.8	-2.1
K01-203-LAI	Kenya	Laikipia	2000	m1	-5.7	-0.8
K01-LAI-191	Kenya	Laikipia	2000	c-(ave)	-2.7	-2.4
OM 2199	Kenya	Naivasha	1966	c	-6.7	-2.9
K00-Nku-233	Kenya	Nakuru	2000	i (dentine)	1.5	-0.4
K00-Nku-234	Kenya	Nakuru	2000	i (dentine)	-3.3	0.3
K00-Tfl-294	Kenya	Ol Bossiyot?	2000	M2	-9.2	2.4
K01-TSW-291	Kenya	Tsavo	1998	C-(ave)	-3.1	-4.3
K99-133-TSV	Kenya	Tsavo	1999	m3	-2.6	-3.1
K99-133-TSV-RM/2	Kenya	Tsavo	1999	m2	-1.8	-2.6
K99-133-TSV-RP/3	Kenya	Tsavo	1999	p3	-1.7	-2.9
K99-156-Tsv	Kenya	Tsavo	1999	c	-4.1	-1.6
K99-157-Tsv	Kenya	Tsavo	1999	c	-4.6	-2.0
K99-158-Tsv	Kenya	Tsavo	1999	c	-5.8	-2.9
K00-Tsv-200	Kenya	Tsavo West	2000	m3	-5.9	-1.8
K00-TSV-226-M/3	Kenya	Tsavo West	2000	m3	-2.8	-3.0
ET-161	Kenya	Turkana	1971	M	-2.1	2.0
ET-162	Kenya	Turkana	1971	M	-0.6	2.2
OM-6102B	Kenya	Turkana	1975	m3	-0.2	4.2
TEC.K89.2	Kenya	Turkana	1974	M	-0.5	2.7
RL-1	Tanzania	Katavi	2000	M	-3.1	-4.6
RL-101	Tanzania	Katavi	2000	M	-1.5	-2.1
RL-102	Tanzania	Katavi	2000	M	-2.2	-0.7
RL-108	Tanzania	Katavi	2000	M	-6.6	-2.3
RL-11	Tanzania	Katavi	2000	M	-3.6	-2.8
RL-15	Tanzania	Katavi	2000	M	-1.4	-3.1
RL-16	Tanzania	Katavi	2000	M	-3.6	-3.6
RL-18	Tanzania	Katavi	2000	M	-3.0	-2.4
RL-19	Tanzania	Katavi	2000	M	-2.4	-3.0
RL-3?	Tanzania	Katavi	2000	M	-4.2	-3.3
RL-31	Tanzania	Katavi	2000	M	-2.1	-4.4
RL-34	Tanzania	Katavi	2000	M	-6.0	-3.7
RL-35	Tanzania	Katavi	2000	M	-4.7	-3.2
RL-37	Tanzania	Katavi	2000	M	-1.9	-2.3
RL-39	Tanzania	Katavi	2000	M	-2.3	-2.5
RL-43	Tanzania	Katavi	2000	M	-4.9	-1.9
RL-44	Tanzania	Katavi	2000	M	-3.2	-2.2
RL-45	Tanzania	Katavi	2000	M	-3.5	-2.6
RL-49	Tanzania	Katavi	2000	M	-0.4	-2.7
RL-5?	Tanzania	Katavi	2000	M	-6.4	-3.8
RL-73	Tanzania	Katavi	2000	M	-2.3	-3.0
OM 2053	Uganda	Murchison	1966	M	-3.3	-0.1
QEP-tusk	Uganda	QEP	1970	c-(ave)	0.0	0.0

U98-QEP-201	Uganda	QEP	1998	m2	-1.9	-0.5
U98-QEP-202	Uganda	QEP	1998	M3	-2.9	-0.8
U98-QEP-203Ap	Uganda	QEP	1998	I (dentine)	-4.1	0.9
U98-QEP-203Bd	Uganda	QEP	1998	I (dentine)	-5.6	-0.4
U98-QEP-208	Uganda	QEP	1998	m1	-6.7	0.4
U98-QEP-210	Uganda	QEP	1998	M3	-4.1	-0.4
U98-QEP-247	Uganda	QEP	1998	m3	-2.1	-0.5

Locality data abbreviations: AS = Arobuko-Sokoke; QEP = Queen Elizabeth Park

Appendix 2. Summary data for stable isotope profiles from modern hippopotamus (*Hippopotamus amphibius*) in this study.

sample (total length: mm)	n	$\delta^{13}C$ ave ±1σ (min, max)	$\delta^{18}O$ ave ±1σ (min, max)
K00-AS-166 (total length = 274 mm)	35	-4.2 ±1.3 (-7.2, -2.7)	-3.0 ±0.6 (-3.9, -1.2)
K00-AS-167 (total length = 467 mm)	35	-2.3±1.3 (-4.5, -0.4)	-3.4 ±0.7 (-4.4, -2.1)
K00-AS-168 (total length = 343 mm)	37	-1.7±1.7 (-6.3, 0.0)	-4.5 ±0.6 (-6.0, -3.4)
K01-Lai-191 (total length*= 480 mm)	24	-2.7±0.3 (-3.3, -2.0)	-2.4 ±1.0 (-4.3, -1.1)
K01-Tsv-291 (total length = 610 mm)	60	-3.1±1.6 (-8.5, -1.3)	-4.2 ±0.8 (-5.2, -0.4)
QEP (total length = 520 mm)	33	0.0±0.5 (-1.0, 0.9)	0.0 ±0.8 (-2.6, 1.4)

* K01-Lai-191. 240 mm of canine was erupted. Total length estimated.