



## OPEN ACCESS

## EDITED BY

Gábor Árpád Czirják,  
Leibniz Institute for Zoo and Wildlife Research  
(LG), Germany

## REVIEWED BY

Stefania Milano,  
Leibniz Institute for Zoo and Wildlife Research  
(LG), Germany  
Stephan Woodborne,  
TAMS Laboratory, South Africa

## \*CORRESPONDENCE

Geoff Koehler  
✉ geoff.koehler@usask.ca

## SPECIALTY SECTION

This article was submitted to  
Ecophysiology,  
a section of the journal  
Frontiers in Ecology and Evolution

RECEIVED 30 September 2022

ACCEPTED 24 January 2023

PUBLISHED 10 February 2023

## CITATION

Koehler G, Schmidt-Küntzel A, Marker L and  
Hobson KA (2023) Delineating origins of  
cheetah cubs in the illegal wildlife trade:  
Improvements based on the use of hair  
 $\delta^{18}\text{O}$  measurements.  
*Front. Ecol. Evol.* 11:1058985.  
doi: 10.3389/fevo.2023.1058985

## COPYRIGHT

© 2023 Koehler, Schmidt-Küntzel, Marker and  
Hobson. This is an open-access article  
distributed under the terms of the [Creative  
Commons Attribution License \(CC BY\)](#). The use,  
distribution or reproduction in other forums is  
permitted, provided the original author(s) and  
the copyright owner(s) are credited and that  
the original publication in this journal is cited, in  
accordance with accepted academic practice.  
No use, distribution or reproduction is  
permitted which does not comply with these  
terms.

# Delineating origins of cheetah cubs in the illegal wildlife trade: Improvements based on the use of hair $\delta^{18}\text{O}$ measurements

Geoff Koehler<sup>1\*</sup>, Anne Schmidt-Küntzel<sup>2</sup>, Laurie Marker<sup>2</sup> and  
Keith A. Hobson<sup>1,3</sup>

<sup>1</sup>NHRC Stable Isotope Laboratory, Environment and Climate Change Canada, Saskatoon, SK, Canada,  
<sup>2</sup>Cheetah Conservation Fund, Otjiwarongo, Namibia, <sup>3</sup>Department of Biology, University of Western Ontario,  
London, ON, Canada

All African felids are listed as vulnerable or endangered according to the IUCN (International Union for Conservation of Nature) Red List of Threatened Species. Cheetahs (*Acinonyx jubatus*) in particular have declined rapidly as a result of human impacts so that development of effective strategies and tools for conservation of this highly vulnerable species, as well as African felids in general, are essential for their survival in the wild. Here we use the oxygen stable isotopic compositions of cheetah hair to determine origins of cheetah cubs destined for the illegal exotic pet trade by associating individual cubs with predicted  $\delta^{18}\text{O}$  isoscape locations. We found that cheetah cubs most likely originated in East Africa, close to the corridors responsible for this aspect of the illegal wildlife trade to the Middle East. Further refinement of these assignments using a two isotope analysis ( $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values) indicate that these cubs were likely sourced in Southern Ethiopia or possibly as far as Tanzania. We also demonstrate that  $\delta^{18}\text{O}$  values in tissues can provide provenance information in cases where results of  $\delta^2\text{H}$  analyses may be obscured by the effects of metabolic routing of nutrients during nursing, starvation, or dehydration. This study demonstrates the utility of stable isotopic tools for conservation and forensic uses for endangered mammalian species.

## KEYWORDS

hydrogen, oxygen, stable isotope, conservation, cheetah, maternal nutrients, metabolic routing

## 1. Introduction

The cheetah (*Acinonyx jubatus*) is one of the most endangered felids in the world. Once wide ranging, they are now only extant in parts of Africa, except for a very small, critically endangered, population of the sub-species *Acinonyx jubatus venaticus* (Asiatic cheetah) in Iran (Farhadinia, 2004). Asiatic cheetahs were hunted to extinction in India, the last three likely to have been killed in 1948 (Velho et al., 2012). The subsequent half century has seen the remaining cheetah distributions contract sharply to only about 10% of their historical range and their numbers similarly decline to only about 7,000 individuals, with over half of the worlds remaining cheetahs resident in southwest Africa (Durant et al., 2017). Other populations are fragmented and small, with numbers of 200 or fewer. The threats to cheetahs are many and, like other wild felids, are primarily the result of anthropogenic pressures. Sub-Saharan Africa has one of the largest growing human populations (Cilliers, 2017) and this has resulted in decimation of wild felid numbers as a consequence of habitat destruction, agricultural expansion, prey reduction, livestock related conflicts, and direct hunting for food, trophies, or curatives (Ripple et al., 2014; Di Minin et al., 2016; Wolf and Ripple, 2017). Cheetahs are in direct competition for prey with other, larger, carnivores, such as lions (*Panthera leo*), leopards (*Panthera pardus*) and hyenas (Hyaenidae) and thus naturally occur in low numbers and are widely dispersed, a situation

that makes conservation by inclusion in national parks difficult. Moreover, their low densities, declining numbers, and solitary nature has resulted in species genetic fragility (O'Brien et al., 1985). These combination of factors make the cheetah, perhaps of all the wild felids, especially vulnerable to existing and growing anthropogenic impacts (Cardillo et al., 2004).

An additional and emerging concern for conservation of cheetahs as well as other charismatic species is the illegal trade of wildlife parts and, more recently, as exotic pets. In some cases, the number of animals affected by the illegal pet market exceeds those from the smuggling of animal parts (Pires, 2012; Daut et al., 2015). Animals entering the exotic pet trade are distributed through illegal criminal organizations and are usually poached from wild populations, although some captive breeding facilities exist (Pires and Moreto, 2011; Dalberg, 2012; Ayling, 2013).

Cheetahs (once called “hunting leopards”) were historically kept and used for hunting in India because they are relatively less ferocious, smaller bodied, and easier to domesticate than other big cats (O'Brien et al., 1986; Bothma and Walker, 1999). Although no longer used for hunting, they are still captured by the modern illegal wildlife trade in Africa whereby cubs are taken alive, usually after killing the mother, and subsequently sold into middle Eastern markets to be kept as “status” pets by the very wealthy (Warchol, 2004; Tricorache et al., 2018; Marker, 2019). Cub mortality during the smuggling process is high with most cubs dying before reaching market (Warchol et al., 2003). Kept as domestic pets, the outlook is similarly grim because cheetahs do poorly in captivity, surviving only a few years without large spatial freedom and specialized diets that mimic those of wild cheetahs (Marker-Kraus, 1997; Tordiffe et al., 2016). Cheetahs are difficult to breed in captivity (O'Brien et al., 1985) so that cubs for the pet trade must be continuously supplied from wild populations, already fragmented and vulnerable. As a result, along with increasing habitat loss, this particular aspect of illegal wildlife trade is now one of the largest current threats to cheetah conservation and survival.

Poached cheetah cubs are funneled through the horn of Africa, across the Gulf of Aden, and into the Arabian Peninsula on their way to illegal markets for exotic pets. The semi-autonomous region of Somaliland, in conjunction with other wildlife agencies, manage to intercept some of these shipments and confiscate a few of the over 300 cheetah cubs that are estimated to move along trafficking routes and into the pet trade every year (Tricorache et al., 2018; Marker, 2019). However, while rescued in Somaliland, the origins of these cubs remain unknown. This is of great concern to conservation and law enforcement agencies, because it is important to know where these cubs are captured so that they may focus their conservation efforts. To address this, we measured the H, O, N, and C stable isotopic compositions of hair from rescued cheetah cubs to try to determine their origins.

The stable isotopic compositions of animal tissues hold great potential for conservation because they may be linked to provenance (Meier-Augenstein et al., 2013; Chesson et al., 2018) or determination of migration patterns of wildlife (Hobson and Wassenaar, 2008). The H and O stable isotopic compositions of hair are related to those of body water, and thus ingested environmental waters (Hobson et al., 1999). Because the H and O stable isotopic compositions of water varies regionally, so do those of animal tissues. Similarly, the carbon isotopic compositions of tissues are related to those of regional vegetation which varies with the relative distribution of C3 and C4

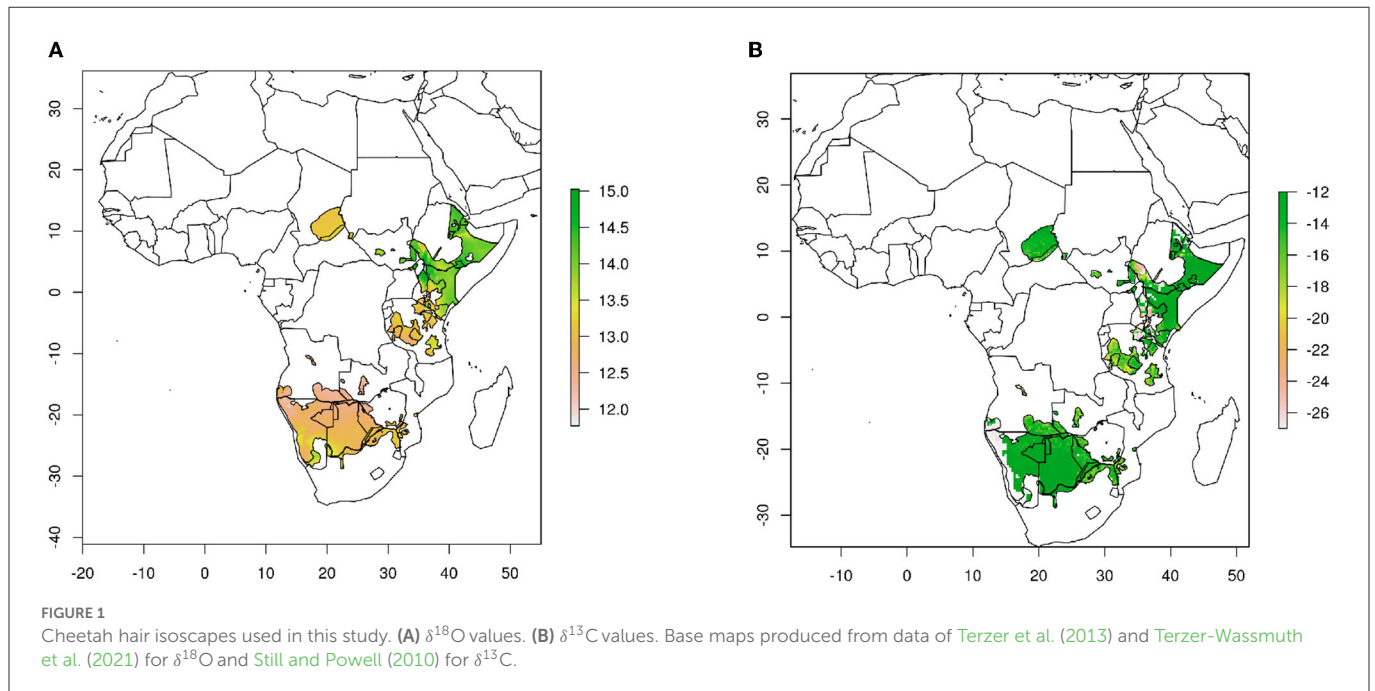
plants and is transferred upwards through the food web. The resulting isotopic landscapes, or “isoscares,” can be used to link organisms with their environment (West et al., 2009; Hobson and Wassenaar, 2018; Vander Zanden et al., 2018). Because of their water conserving nature and obligate carnivore physiology, isotopic linking of felids to environmental water and thus location was not considered feasible (Pietsch et al., 2011). However, a recent study by Koehler and Hobson (2019) has determined that a relationship between environmental water and felid hair does indeed hold for domestic and wild cats and therefore may be used for provenance estimation. A remaining issue pertains to the use of cub hair because young mammals may be influenced by maternal nutrient inputs through nursing and such maternal effects have not been previously considered in isotopic assignments. Our objective, then, was to use isotopic techniques to establish the provenance of cheetah cubs confiscated in Somaliland and to explore means to resolve the maternal nutrient transfer issue. Ultimately though, it is our hope that this proof of concept can be further expanded to other terrestrial mammals, particularly those that are currently threatened by illegal activities.

## 2. Methods

Hair samples (*ca.* 20 mg) were collected from 2015 to 2021 during veterinary health checks from lawfully confiscated cheetah cubs ( $n = 96$ ) after rescue in Somaliland. The ages of cubs ranged between 3 and 7 months, as determined by veterinary assessments after rescue. All hair samples were shipped to the NHRC Stable Isotope Laboratory under CITES export permits 0061077 and 0063888 and imported with Canadian CITES import permit 21CA01252/CWHQ. This study was approved by the University of Saskatchewan Research Services and Ethics Office, RSEO Reference number 003Exempt2020.

### 2.1. Stable isotope analysis

Stable isotopic compositions of all hair samples were performed at the NHRC Stable Isotope Laboratory of Environment and Climate Change Canada in Saskatoon, SK, Canada. Prior to analyses, hair samples were cleaned of adherent debris and any surface oils were removed by rinsing in 2:1 chloroform:methanol. Hair was homogenized to powder with a ball grinder (Retsch model MM-301, Haan, Germany). The root end of the hairs were discarded as these will reflect the most recent growth and may include hair grown in captivity prior to rescue. For hydrogen and oxygen, our approach involved the measurement of both  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values with the same analytical run (i.e., both  $\text{H}_2$  and  $\text{CO}$  gases were analyzed from the same pyrolysis) (Hobson and Koehler, 2015). Samples and standards were weighed to  $350 \pm 20 \mu\text{g}$  in silver capsules and analyzed using a Delta V Plus IRMS system (Thermo Finnigan, Bremen, Germany) equipped with a Costech Zero-Blank autosampler. The helium carrier gas rate was set to 120 mL/min. We used a HTC 1.5 m 0.25 inch 5 Å molecular sieve (80–100 mesh) GC column. The glassy carbon reactor was operated at a temperature of 1,400°C, and the GC column temperature was set to 90 °C. After separation, the gases were introduced into a Delta V plus isotope-ratio mass spectrometer *via* a ConFlo IV interface (Thermo Finnigan, Bremen, Germany). The eluted  $\text{N}_2$  was flushed to waste by withdrawing the CF capillary from the ConFlo interface. We



used Environment Canada keratin reference standards CBS (Caribou hoof) and KHS (Kudu horn) to calibrate sample  $\delta^2\text{H}$  (−197 and −54.1 per mil, respectively) and  $\delta^{18}\text{O}$  values (+2.50 and +21.46 per mil, respectively Qi et al., 2011). This normalization with calibrated keratins also corrects for any hydrogen isotope measurement artifact caused by production of HCN (Gehre et al., 2015) in the glassy carbon reactor as described by Soto et al. (2017). Based on replicate ( $n = 10$ ) within-run measurements of keratin standards and from historical analyses of an in-house QA/QC reference (SPK keratin), sample measurement error was estimated at  $\pm 2$  per mil for  $\delta^2\text{H}$  and  $\pm 0.4$  per mil for  $\delta^{18}\text{O}$ . All H results are reported for nonexchangeable H and for both H and O in the standard delta notation, normalized on the Vienna Standard Mean Ocean Water—Standard Light Antarctic Precipitation (VSMOW-SLAP) scale.

For carbon and nitrogen isotope analyses, we weighed 1 mg of ground hair into precombusted tin capsules. Encapsulated hair was combusted at 1,030°C in a Carlo Erba NA1500 elemental analyser. The resulting  $\text{N}_2$  and  $\text{CO}_2$  were separated chromatographically and introduced to an Elementar Isoprime isotope ratio mass spectrometer (Langensfeld, Germany—[www.elementar.de](http://www.elementar.de)). We used two calibrated in-house reference materials to normalize the results to VPDB and AIR: BWBIII keratin ( $\delta^{13}\text{C} = -20.18$ ,  $\delta^{15}\text{N} = 14.31$  per mil, respectively) and PRCgel ( $\delta^{13}\text{C} = -13.64$ ,  $\delta^{15}\text{N} = 5.07$  per mil, respectively). Precisions as determined from both reference and sample duplicate analyses and from within-run analyses of QA/QC bovine gelatin (BVgel) were  $\pm 0.1$  per mil for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . For both HO and CN stable isotope analyses, most samples were run in duplicate.

## 2.2. Assignment to origin

We used geographical assignment algorithms as described in Van Wilgenburg and Hobson (2011). Briefly, we used rastered  $\delta^{18}\text{O}$  hair-specific isoscapes as a basis for predicting origins of cheetah

cubs produced from the rescaling factors measured for known-origin cats across North America (Figure 1A) (Koehler and Hobson, 2019). These hair  $\delta^{18}\text{O}$  isoscapes were then imported into custom R scripts using the `rgdal`, `sp` and `raster` packages and used to plot 2-D spatial data in map form, and perform spatial statistics on the raster surfaces. We used current cheetah range shapefiles from the IUCN, but did not include the ranges of two critically endangered (i.e., < 30 individuals) cheetah subspecies, the Asiatic cheetah (*A. j. venaticus*) and the North African cheetah (*A. j. hecki*). We then applied a normalized probability density method (Hobson et al., 2009) to estimate the probability that individual cells in the calibrated felid isoscape represented a potential origin for each cub at the 67% (i.e., 2:1 odds ratio) confidence level. Digital file manipulation and assignment to origin analyses were conducted using multiple packages including “`raster`” v.3.6-3 (Hijmans and Van Etten, 2015) and “`maptools`” v. 1.1-4 (Bivand and Lewin-Koh, 2015) in the R statistical computing environment v.4.1.2 (R Core Team, 2021).

For carbon isotopes, we used  $\delta^{13}\text{C}$  isoscapes for Africa produced from the theoretical spatial distribution of plants obtained from C3/C4 plant abundance remote sensing maps (Still and Powell, 2010; Hobson et al., 2012). We did not use a rescaling factor because previous studies have indicated that cheetah fur has similar  $\delta^{13}\text{C}$  values to those of food (Voigt et al., 2014), whereas an enrichment of 1 to 4 per mil is measured for other felids (Parnig et al., 2014; Montanari and Amato, 2015) (Figure 1B).

## 3. Results and discussion

The  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  values of hair from 96 cheetah cubs are shown in Table 1. Values of  $\delta^{15}\text{N}$  of these cubs ranged from +8.6 to +16.9 per mil, and are significantly higher (mean = 13.4,  $\sigma = 1.7$  per mil) than those reported values for African lions or leopards (Codron et al., 2007). Adult cheetahs from Namibia have similar  $\delta^{15}\text{N}$  values (+12.3 per mil) to those of other African felids (Voigt et al., 2014), largely because most felid species tend to prey on

TABLE 1 Nitrogen, carbon, hydrogen, and oxygen stable isotopic compositions of hair from cheetah cubs confiscated from the illegal pet trade, Somaliland.

Name	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$	Name	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$
Cloud	-21.5	14.1	-45	16.4	Kiana	-13.2	10.9	-15	14.8
Star	-20.7	14.9	-35	15.4	Serge	-20.9	11.8		
Mist	-22.1	13.6	-44	16.1		-22.1	12.9		
Moonlight	-21.1	14.1	-49	16.6	Amiin			-6	15.1
Sunshine	-23.1	15.4	-61	17.1	Zelda	-22.4	13.8	-27	14.2
Libbo	-21.1	16.7	-14	15.2	Amiir	-22.2	16.9	-12	15.4
Lyaru	-21.0	16.3	-3	15.9	Moxie	-19.1	12.8	-37	14.5
Leo	-21.2	16.4	-7	15.3	Zero	-19.7	12.8	-7	16.6
Galol	-22.2	14.6	-25	15.9	Johnny-2,750	-18.5	13.1	-32	14.7
Kidi	-22.3	14.4	-31	14.5	Olivia			-16	15.6
Kulan	-22.3	14.7	-23	16.6	Sol	-21.1	11.8	-24	17.5
Lion	-19.5	10.5	-34	13.5	Fush	-22.7	14.7	-22	15.2
Libra	-14.7	12.7	-43	15.0	Rocket	-22.1	14.1	-27	13.9
Abbo	-21.2	14.4	-6	16.0	Mischief	-21.5	14.9	-5	16.0
Mariam	-19.1	14.7	-10	15.5	Phil	-21.1	14.1	-39	15.6
Libbo	-20.3	13.0	-36	13.2	Frigga	-20.1	13.3	3	16.5
Leo	-20.6	13.8	-25	13.7	Freya	-20.0	13.4	-25	17.1
Lyam	-20.5	12.8	-41	13.6	Sif	-20.5	11.4	-12	16.9
Kalli	-21.5	14.3	-13	15.5	Magan	-20.1	14.1	3	15.5
Guhad	-19.2	14.8	-2	16.6		-21.7	14.1	-44	15.2
Ayanna	-20.4	14.0	-16	16.3	Link	-20.4	15.2	-9	14.9
Kurro	-21.3	14.4	-7	16.1	Duma	-18.5	12.3	-28	13.9
Dominic	-13.0	11.5	-38	13.3		-21.8	14.7	-45	16.2
Savanna	-18.5	12.4	2	19.7	Teresa	-20.1	14.8	2	16.4
Smartman	-13.2	10.8	-28	14.3	Yasir	-20.3	14.9	8	15.8
Polly	-13.3	10.1	-31	14.3	Vicki 2	-19.2	13.6	-2	16.8
B2	-13.1	8.6	-38	12.9	Major	-19.9	13.2	-33	15.6
Little C	-12.8	9.1	-41	16.8	Koya	-19.9	14.0	-49	14.1
Ron	-12.8	9.5	-32	11.9	Janet	-18.3	13.9	-27	15.6
Hermione	-13.6	10.2	-38	14.4	Faduma	-18.4	13.4	-29	15.1
Max	-13.0	10.6	-45	13.7	Meeko	-18.3	13.7	-42	13.8
Thor	-12.8	11.0	-35	13.5	Shamsi	-20.7	14.0	-33	16.1
West	-12.9	11.2	-42	14.3	DJ	-18.3	14.1	-32	13.9
Ben	-12.8	10.9	-39	13.6	Micket	-18.4	13.5	-33	14.6
Mike	-12.9	11.3	-39	14.3	James-4269	-18.2	13.5	-35	15.0
Darwin	-13.0	10.7	-31	14.0	Idris	-20.0	14.5	-10	15.6
Nico	-13.0	10.8	-29	13.8	Elba	-19.6	13.8	-7	17.6
Kayla	-12.7	11.8	-31	12.9	Astur	-20.4	14.3	1	12.8
Merlot	-13.3	10.8	-21	15.7	Kaiir	-20.7	14.7	9	13.8
Romeo	-14.5	11.1	-11	15.8	Jaleelo	-19.9	14.0		
Khayjay	-13.0	11.3	-18	14.9	Galiil	-20.5	15.0	-4	14.9
Rosy	-13.3	11.2	-15	15.4	Rajo	-18.5	13.4	-21	12.3

(Continued)

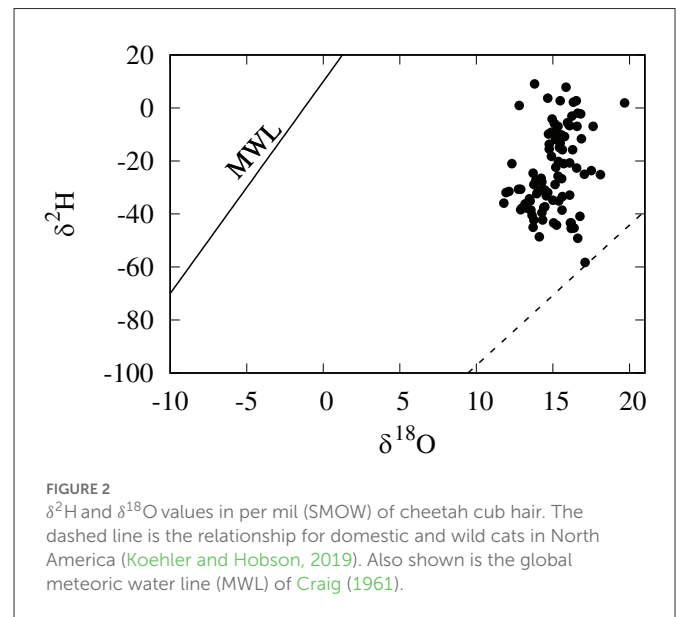
TABLE 1 (Continued)

Name	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$	Name	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$
Solo	-13.1	11.1	-10	15.3	Cizi	-18.6	13.0	4	14.7
Amani	-13.2	10.6	-10	14.7	Emmeh	-18.5	13.8	-38	13.0
Harry	-13.6	11.4	-12	15.2	Margarita	-18.4	13.8	-31	12.2
Blondeman	-13.8	11.4	-43	16.2	Bagheer	-18.4	13.0	-31	12.7
Phoenix	-14.6	11.4	-21	16.1	Darth	-18.4	13.2	-36	11.8
Fossey	-14.5	11.5	-26	15.3	Dead cheetah	-19.7	13.2	-20	15.4

ungulates or other small bodied herbivores (Eaton, 1970; Mills, 1984). The most likely reason for the observed high  $\delta^{15}\text{N}$  values in these cheetah cubs is the well known trophic effect from nursing young to offspring, although other effects such as malnutrition, cannot be ruled out. Adult mammals isotopically integrate their environment through coupling of H, O, C, and N isotopes into tissues by direct ingestion of food and water (Hobson and Wassenaar, 2018). For nursing young, however, this relationship is complicated because young mammals obtain a significant amount of environmental water and nutrients indirectly through maternal milk thereby placing young at a higher trophic position (i.e., higher  $\delta^{15}\text{N}$  values) than their mothers. The high lipid content of milk also tends to drive cub tissue  $\delta^{13}\text{C}$  values lower than those formed after weaning.

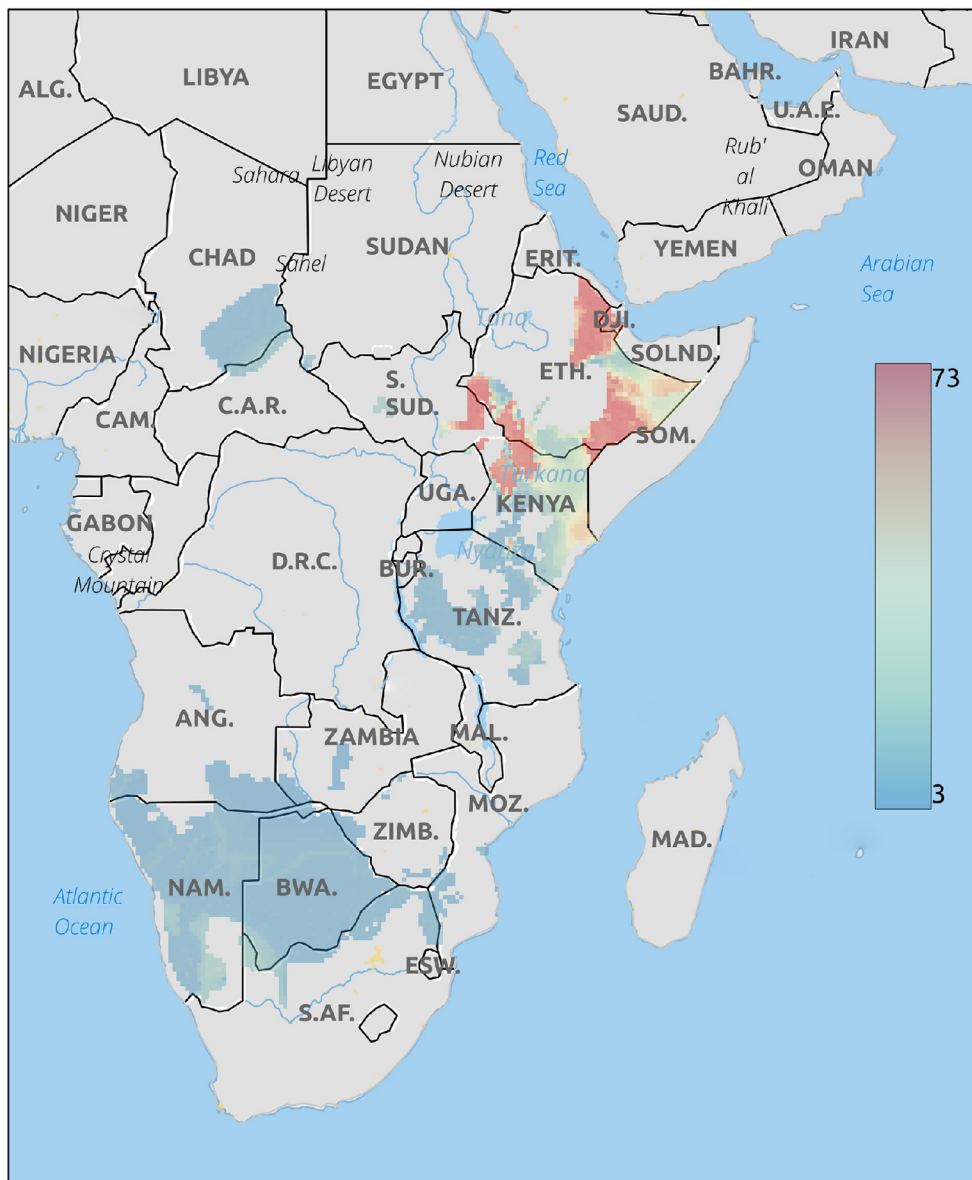
With the exception of humans, there is very little information in the literature on the relative discrimination of stable isotopes between tissues of mothers and nursing young. A few studies have compared the stable isotopic compositions of herbivores, generally cows (*Bos taurus*), with diet (Kornexl et al., 1997; Camin et al., 2008), or location (Chesson et al., 2010), mostly for detection of food adulteration. Jenkins et al. (2001) determined that isotopic differences between maternal and offspring plasma were variable and species dependent. Lipids are generally  $^{13}\text{C}$  depleted relative to other animal tissues (Tieszen et al., 1983) and also to the rest of the components in milk (Melzer and Schmidt, 1987; Wilson et al., 1988), so that we would expect tissues of nursing offspring to have lower  $\delta^{13}\text{C}$  values than those of the mother. The low  $\delta^{13}\text{C}$  values of many of these cheetah cubs (Table 1) compared with Namibian cheetahs ( $\delta^{13}\text{C} = -14.8$  per mil Voigt et al., 2014) are consistent with this, however the  $\delta^{13}\text{C}$  values of the maternal diet are unknown so it is impossible to make comparisons.

Polar bear (*Ursus maritimus*) cubs have higher  $\delta^{15}\text{N}$  values and lower  $\delta^{13}\text{C}$  values than do adults from the same subpopulations (Polischuk et al., 2001; Koehler et al., 2019). Hair  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  values of polar bear cubs are typically lower than those of adults (Koehler et al., 2019), most likely because of the high lipid content of maternal milk with correspondingly low  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  values. The amount of the differences in these isotopic compositions is likely dependent on the relative ages of cubs, and thus amount of nursing, and of the lipid content of the milk. The fat content of cheetah milk (64 g/kg) is lower than milk from polar bears (350 g/kg Derocher et al., 1993) and slightly lower than lions or domestic cats (*Felis catus*) (De Waal et al., 2004; Osthoff et al., 2006). Therefore compared to polar bears, we would certainly expect a smaller or perhaps no difference in  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  values between adult cheetahs and cheetah cubs. Ultimately, though, there is no way to determine this other than using controlled diet studies.



Values of hair  $\delta^2\text{H}$  for these Cheetah cubs were more variable and positive than we anticipated, ranging from  $-61$  to  $+9$  per mil. Because the  $\delta^2\text{H}$  values of lipids are much lower than that of other macromolecules (Sessions et al., 1999; Sachse et al., 2012), we expected the  $\delta^2\text{H}$  values of nursing cub hair to be lower than those of adult cats relative to their oxygen isotopic compositions, however this was clearly not the case (Figure 2). One possible explanation for this observation is that the relative enrichment in  $^2\text{H}$  in hair in cubs is the result of a trophic effect between mother and cub. Because these cubs were generally young (3–7 months), it is likely that a portion of the hair was grown before birth and prior to nursing and introduction of the lipid-rich milk diet.

For placental mammals, *in utero* nutrient and gas exchange occurs across the fetal villi without exchange of blood. In humans, fetal amino acid concentrations are generally higher than maternal levels (Cetin et al., 1992), reflecting an active transport mechanism across the placental membranes. It seems unlikely that active transport of large molecules such as amino acids will result in significant isotopic fractionation, and thus we would expect the familiar trophic effect *in utero* that is observed in nursing young, and is consistent with the  $\delta^{15}\text{N}$  values of cubs in this survey. This has been observed in humans, where  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of newborn infant hair were higher than those of the mother (De Luca et al., 2012). For hydrogen isotopes, a trophic enrichment of 30 to 50 per mil is predicted from mechanistic models (Magozzi et al., 2019) and



**FIGURE 3**  
 Probable origins of cheetah cubs rescued from the illegal pet trade based on  $\delta^{18}\text{O}$  isoscapes. Legend is the number of individuals assigned to a pixel based on the odds ratio criterion used.

observed in a few studies (Birchall et al., 2005; Topalov et al., 2013). If this is the case for cheetahs, the variability in hydrogen isotope enrichment in cheetah cubs may be related to their variable ages where the  $\delta^2\text{H}$  values of older cubs would be more negative, reflecting more a contribution of nursing in the hair. Unfortunately, we do not have exact ages for the cubs and, historically, it is has been difficult to separate a hydrogen isotopic trophic effect from other metabolic and dietary processes with any certainty (reviewed by Vander Zanden et al., 2016).

Another complication with the cheetah cubs we examined was that they are usually extremely malnourished and dehydrated when rescued. Starvation in humans generally increases the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of hair by recycling of proteins within the body pool (Hatch et al., 2006; Mekota et al., 2006), a phenomenon also noted in birds (Hobson et al., 1993; Cherel et al., 2005). Nutritional and water

stress has been suggested as the mechanism for large increases in the  $\delta^{15}\text{N}$  values in bone collagen in African herbivores (Ambrose and DeNiro, 1986). For hydrogen and oxygen isotopes, however, there appears to be very little in the literature on the effects of starvation on the isotopic compositions of animal protein. For dehydration, it seems intuitive that evaporation concurrent with respiration and diffusive water loss would result in higher  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of the body water pool with corresponding increases of those of hair. Indeed, McKechnie et al. (2004) observed that feathers of water-stressed Rock Doves (*Columba livia*) were enriched in  $^2\text{H}$ , similar to previous observations in humans (Schoeller et al., 1986). However, the picture is not as clear for oxygen isotopes for which there are very few measurements. Storm-Suke et al. (2012) observed similar hydrogen isotope results in Japanese quail (*Coturnix japonica*) as was observed for Rock Doves by McKechnie et al. (2004),

but did not see any differences for oxygen isotopes. Ultimately, though, whether it can be attributed to maternal trophic effects, malnutrition, or dehydration, the relatively high  $\delta^2\text{H}$  values of these cubs remain enigmatic.

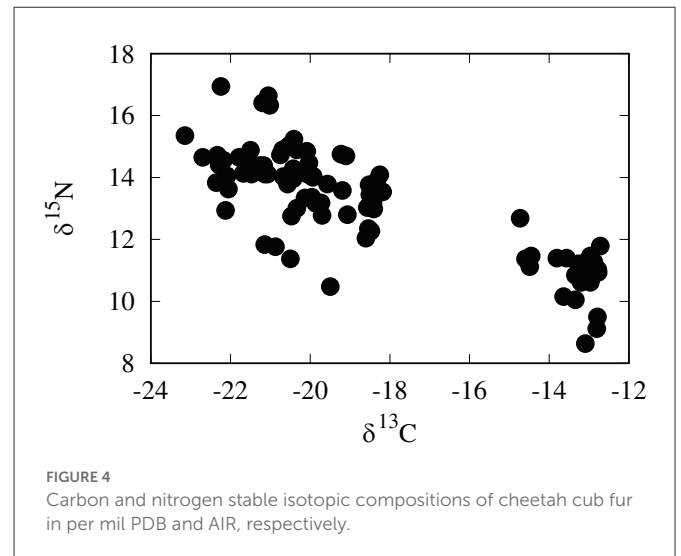
For geographical assignments, tissue  $\delta^2\text{H}$  values in consumers are generally preferred over  $\delta^{18}\text{O}$  values because of their greater range and smaller relative error (Hobson and Koehler, 2015). But, because of all the aforementioned uncertainties involved with the use of  $\delta^2\text{H}$  values of hair from cheetah cubs, we decided to avoid these complexities altogether and instead use hair  $\delta^{18}\text{O}$  values for geographical placement. The  $\delta^{18}\text{O}$  values of adult and cub hair from polar bears from the same locations did not show any significant difference, although there were large differences in  $\delta^2\text{H}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{13}\text{C}$  values (Koehler et al., 2019). This is most likely because lipids in maternal milk contain little oxygen and the  $\delta^{18}\text{O}$  values of milk protein is similar to those of the maternal diet. Because oxygen is more weakly routed from diet to consumer tissues, it is likely that the isotopic compositions of oxygen in these tissues will be less confounded by environmental and dietary considerations than hydrogen (Bowen et al., 2009; Soto et al., 2011; Wolf et al., 2015), and thus will more closely reflect the isotopic compositions of environmental water. It is currently unknown whether malnutrition or dehydration can cause changes to the  $\delta^{18}\text{O}$  values of mammal hair.

### 3.1. Geographical origins of cheetah cubs

Cheetah females are polyestrous and are thus non-seasonal breeders (Crosier et al., 2018, 2022). As a result, cubs are captured in the illegal trade throughout the year, with a slight bias to dry season (Jul-Aug), perhaps as a result of easier transport along illegal trade routes. Assuming the cubs are, on average, 6 months old, this indicates they were likely born during a wet season in East Africa. Here, with the exception of northern Ethiopia, the majority of precipitation falls during two wet seasons (Levin et al., 2009). In this case, the mean annual oxygen isoscape is appropriate because the bulk of the precipitation occurs in the wet seasons and the isoscape is representative of this. Considering the wide range of ages of the cubs and that cubs can be taken at any time during the year, it is impossible to associate a particular season with most of the cubs with any accuracy.

For geographic placement, we cannot assume a single batch of cubs captured at a single time. In reality, cubs were captured in small batches (1 to a few) from 2015 to 2021. We also have to consider the isotopic integration that occurs through the foodweb which will tend to average out wet season-dry season differences in stable isotopic compositions of hair. While prey species are more closely coupled to the underlying precipitation isoscape, they will also tend to average out yearly precipitation to some extent based on growth characteristics and seasonal isotope patterns in plants. Considering all of this, we treated all 96 cubs as a single batch of time-integrated samples and assigned them to origin using the mean amount averaged oxygen isoscape. This approach provides an estimation of the total geographic range of cub origins, an important consideration for law enforcement and conservation.

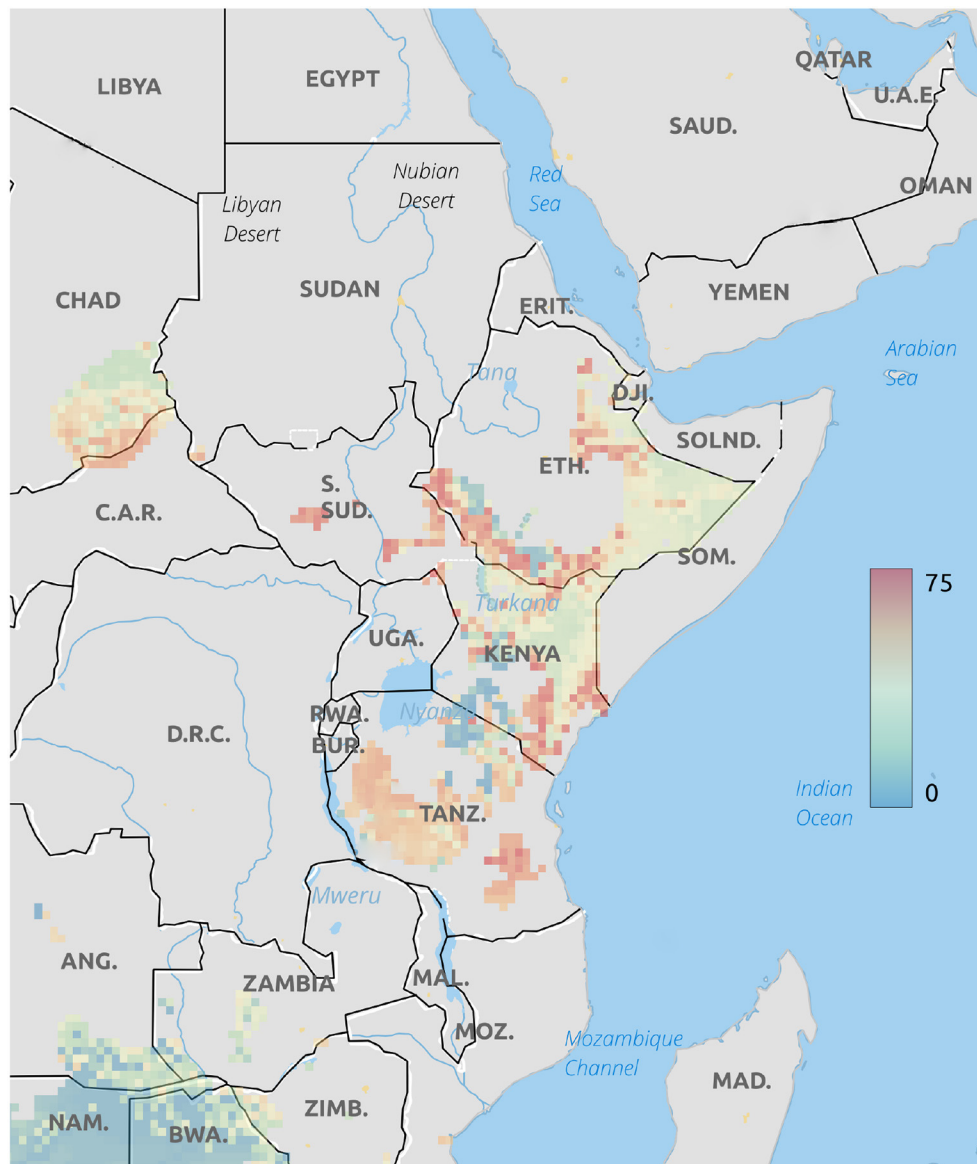
Because oxygen isotopic compositions are rarely used, it is important to take into account the error envelope involved in geographical placement of cheetah cubs. If we examine the standard error of  $\delta^{18}\text{O}$  values reported for the RCWIP dataset and translate



those to the cheetah isoscape as seen on Figure 1A, oxygen isotopic compositions of cheetah fur are only expected to vary by about 0.4 per mil at any single location in east Africa, similar to our measurement error for  $\delta^{18}\text{O}$  values. Our measured range of  $\delta^{18}\text{O}$  values in cheetah fur exceeds 5 per mil (Table 1) so that we are confident that geographic placement is outside the analytical error. In addition, the assignment algorithm takes this error into account so that the oxygen isotopic composition of any cub that matches the isoscape does so at the 2:1 odds ratio or the 67% ( $1\sigma$ ) confidence interval.

The Geographical placement of cheetah cubs based on  $\delta^{18}\text{O}$  values of hair are shown in Figure 3. This analysis places these cubs in northwestern Kenya, northern Ethiopia, or southern Ethiopia close to the border with Somalia. These areas, particularly along the Somali border are close to the expected routes for the illegal trade in live cubs (Marker, 2019).

Although regionally accurate, assignments of cheetah cubs to geographic origin are fairly broad based only on  $\delta^{18}\text{O}$  values. To further refine these assignments we examined the utility of a multivariate placement using both  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values. Values of  $\delta^{13}\text{C}$  of cheetah cubs range from  $-22.7$  to  $-12.7$  per mil and show a clear bimodal distribution (Figure 4 and Table 1). While relatively high  $\delta^{13}\text{C}$  values of cubs were expected from the east African savannahs where the C4 grasses dominate in the warm, arid lowlands (Tieszen et al., 1979b), many cubs had unexpectedly low  $\delta^{13}\text{C}$  values. At first, we attributed these low  $\delta^{13}\text{C}$  values of these cubs to consumption of a high lipid diet during nursing, but on further analysis this seems unlikely. Polar bear cubs have  $\delta^{13}\text{C}$  hair values that are only 1–3 per mil lower than those of adults (Koehler et al., 2019) concurrent with very high concentrations of lipids in maternal milk. Cheetahs have lower amounts of lipids in maternal milk, so it seems unlikely that there would be a similar large negative offset in  $\delta^{13}\text{C}$  values between cheetahs adults and nursing young. If ingestion of lipid rich milk were responsible for the low  $\delta^{13}\text{C}$  values observed, we would also expect a similar decrease in  $\delta^2\text{H}$  values, but we see the opposite. Furthermore, fur from free ranging Namibian cheetahs have  $\delta^{13}\text{C}$  values about  $-14.8$  per mil (Voigt et al., 2014), very close to the  $-12$  to  $-13$  per mil predicted from the African carbon isoscape (Figure 1B), which reflects the abundance of C4 plants and therefore C4-eating prey in tropical grasslands. Feeding



**FIGURE 5**  
 Multivariate isotope assignment of cheetah cubs to location based on a  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values. Legend is the number of individuals assigned to a pixel based on the odds ratio criterion used.

studies of adult cheetahs also indicate there only minor differences in  $\delta^{13}\text{C}$  values between cheetahs and food sources (Voigt et al., 2014). These observations suggest that, overall, the isotopic discrimination of carbon isotopes is close to 0 per mil between cheetahs and their environment. Therefore, if the consumption of a lipid rich milk is responsible for the low  $\delta^{13}\text{C}$  values measured in these cubs, we would expect that hair from cheetah cubs should have  $\delta^{13}\text{C}$  values around -15 per mil.

An important consideration is that accurate transfer of carbon isoscapes based on terrestrial foodwebs have never been ground-tested for mammals. Stable carbon isoscapes are based on the predicted spatial proportions of C4 and C3 plants obtained from remote sensing and, as a result, there is a uncertainty differentiating between grasses and non-tree herbaceous layers in the present carbon isoscapes (Still and Powell, 2010). In theory, carnivores

should integrate the total carbon isotopic compositions of these relative proportions and therefore should closely reflect their  $^{13}\text{C}$  environment. Herbivores tend to be selective and can be classified as grazers (C4-eating) or browsers (C3-eating). Therefore, provided that browsers and grazers are in proportion to the relative proportions of C3 to C4 plants and that predators are non-selective, the carbon isotopic compositions of predator tissues should be similar to those of the carbon isotope biome in which they inhabit.

In practice, however, it is undoubtedly more complicated. The  $\delta^{13}\text{C}$  values of tissues in carnivores will be a function not just of the underlying carbon isoscape, but will also be compounded by trophic discrimination factors, hunting behavior, and foraging behavior of prey. For higher level taxa, such as felids or canids, we would also expect a variation of hunting behaviors depending on availability and/or individual preferences. Indeed, we know that cheetahs can



develop strategies to overcome particular prey animals (Mills, 1984; Marker et al., 2003) and this may account for the clear exploitation of either grazers or browsers as prey in a mixed tree/grass savannah (Figure 4). Higher  $\delta^{15}\text{N}$  values in cub hair with low  $\delta^{13}\text{C}$  values seem to confirm this as mixed feeding gazelles have relatively high  $\delta^{15}\text{N}$  values compared to grazers (Ambrose and DeNiro, 1986).

Impalas (*Aepyceros melampus*), Grants gazelles (*Nanger granti*) and Thompsons gazelles (*Eudorcas thomsonii*) comprise a large part of the diet of East African cheetahs (Eaton, 1970), all of which are mixed feeders wherein their diet consists of both C3 and C4 plants and varies temporally depending on species and the season (Spinage et al., 1980). Grants gazelles, which tend to browse more than they graze, occur in Ethiopia and South Sudan, whereas Thompsons gazelles and impalas, which can graze or browse, are more common in Kenya and Tanzania (Arctander et al., 1996). Consequently the  $\delta^{13}\text{C}$  values of the stomach contents of these prey species can vary from average values of  $-21.9$  per mil for Grants gazelles to  $-17$  per mil for Thompsons gazelles, with Impalas averaging about  $-19$  per mil (Tieszen et al., 1979a). Therefore, it is likely that the bimodal  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of these cubs simply reflect prey preference of the mother and thus are only loosely coupled to the underlying carbon isoscape.

An alternate, but less likely, explanation is that the C3 group of cubs are younger because they were captured closer to the trafficking routes in the Horn of Africa in the mixed savannah proximal to the Ethiopian highlands. In this case, the low  $\delta^{13}\text{C}$  values will be augmented by lipids in milk and the elevated  $\delta^{15}\text{N}$  values may be partially the result of maternal trophic effects.

The dual-isotope model places the origins of these cubs primarily in south western Ethiopia, as do the assignments based only on  $\delta^{18}\text{O}$  values (Figure 5). However, the abundance of low  $\delta^{13}\text{C}$  values in cheetah cub hair suggests many cubs originated in the savannah highlands where most of the forbs, shrubs, and trees are C3 types or, possibly, as far south as the great rift region of Tanzania. Considering all of the complexity involved in transfer of the carbon isotopes into predator tissues, however, this analysis cannot be taken as diagnostic and is of limited usefulness. While it is unlikely that these cubs could survive transport over the  $>1,000$  km between Tanzania and Somalia, it is certainly possible that many could have been captured in southwestern Ethiopia where, presumably, Grants gazelles would make up a larger part of the adult diet.

## 4. Conclusions

Based on  $\delta^{18}\text{O}$  values only, it is evident that cheetah cubs destined for the illegal pet trade are sourced from East Africa, primarily northern Ethiopia, western Kenya, and along the Somali border with Kenya. Further refining these placements by using a two isotope multivariate assignment suggests that it is possible that these cubs could have been sourced as far south as Tanzania. The two isotope analysis is, at best, uncertain because of complexities relating the underlying carbon isoscape to the terrestrial foodweb. It seems obvious that geographic placement of cheetah cubs places them proximal to the illegal trade routes. However, this approach also demonstrates the general usefulness of stable isotopic placement for forensic analyses in terrestrial mammals, particularly the carnivores. In addition, it is our strong recommendation that oxygen isoscapes be considered for geographic placement in cases where the normal

transfer of nutrients into tissues are complicated by the effects of nursing or nutritional stress.

Moving forward, it is our hope that these isotopic techniques can be developed into a robust analytical protocol that can be used by law enforcement and conservation agencies to address the many aspects of the illegal wildlife trade, not just for cheetahs, but for other vulnerable or endangered species. To this end, the addition of other intrinsic markers, such as genetic or chemical information, will help to refine geographical placement or identification of individuals for forensic applications. In the short term, automatic sampling and archiving of all confiscated illegally captured cheetahs is needed. Ultimately though, successful development and use of stable isotopic techniques will require a concerted effort from scientists, law enforcement, conservation groups, and policy makers. Like cheetahs, many of the world's iconic species are currently under threat and these novel avenues for wildlife conservation may contribute to their very survival.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## Ethics statement

This animal study was reviewed and approved by the University of Saskatchewan, 003Exempt2020.

## Author contributions

GK and KH contributed to conception and design of the study. AS-K and LM orchestrated sampling and logistics. GK collected the data, performed the statistical analysis, and wrote the first draft of the manuscript. GK and KH contributed equally to manuscript revision and all authors approved the submitted version.

## Funding

This study was funded by Environment and Climate Change Canada.

## Acknowledgments

The contributions of Andrei Mihalca, Carolyn Farquhar, and Patricia Tricorache were essential for sample collection, CITES permits, and logistics. The help and guidance of the Cheetah Conservation Fund was invaluable and we look forward to working with them in the future. We thank Chantel Gryba and Jessica Fehr for sample preparation and for assistance in stable isotope analyses.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## References

- Ambrose, S. H., and DeNiro, M. J. (1986). The isotopic ecology of east african mammals. *Oecologia* 69, 395–406. doi: 10.1007/BF00377062
- Arctander, P., Kat, P. W., Aman, R. A., and Siegmund, H. R. (1996). Extreme genetic differences among populations of gazella granti, grant's gazelle, in kenya. *Heredity* 76, 465–475. doi: 10.1038/hdy.1996.69
- Ayling, J. (2013). What sustains wildlife crime? rhino horn trading and the resilience of criminal networks. *J. Int. Wildlife Law Policy* 16, 57–80. doi: 10.1080/13880292.2013.764776
- Birchall, J., O'Connell, T. C., Heaton, T. H. E., and Hedges, R. E. M. (2005). Hydrogen isotope ratios in animal body protein reflect trophic level. *J. Anim. Ecol.* 74, 877–881. doi: 10.1111/j.1365-2656.2005.00979.x
- Bivand, R., and Lewin-Koh, N. (2015). maptools: Tools for reading and handling spatial objects. *R package version 0.8–39*.
- Bothma, J. d. P., and Walker, C. (1999). "The cheetah," in *Larger Carnivores of the African Savannas* (Springer), 92–115.
- Bowen, G. J., Ehleringer, J. R., Chesson, L. A., Thompson, A. H., Podlesak, D. W., and Cerling, T. E. (2009). Dietary and physiological controls on the hydrogen and oxygen isotope ratios of hair from mid-20th century indigenous populations. *Am. J. Phys. Anthropol.* 139, 494–504. doi: 10.1002/ajpa.21008
- Camin, F., Perini, M., Colombari, G., Bontempo, L., and Versini, G. (2008). Influence of dietary composition on the carbon, nitrogen, oxygen and hydrogen stable isotope ratios of milk. *Rapid Commun. Mass Spectrometry* 22, 1690–1696. doi: 10.1002/rcm.3506
- Cardillo, M., Purvis, A., Sechrest, W., Gittleman, J. L., Bielby, J., and Mace, G. M. (2004). Human population density and extinction risk in the world's carnivores. *PLoS Biol.* 2, e197. doi: 10.1371/journal.pbio.0020197
- Cetin, I., Marconi, A., Corbetta, C., Lanfranchi, A., Baggiani, A., Battaglia, F., et al. (1992). Fetal amino acids in normal pregnancies and in pregnancies complicated by intrauterine growth retardation. *Early Hum. Dev.* 29, 183–186. doi: 10.1016/0378-3782(92)90136-5
- Cherel, Y., Hobson, K. A., Bailleul, F., and Groscolas, R. (2005). Nutrition, physiology, and stable isotopes: new information from fasting and molting penguins. *Ecology* 86, 2881–2888. doi: 10.1890/05-0562
- Chesson, L. A., Barnette, J. E., Bowen, G. J., Brooks, J. R., Casale, J. F., Cerling, T. E., et al. (2018). Applying the principles of isotope analysis in plant and animal ecology to forensic science in the americas. *Oecologia* 187, 1077–1094. doi: 10.1007/s00442-018-4188-1
- Chesson, L. A., Valenzuela, L. O., O'Grady, S. P., Cerling, T. E., and Ehleringer, J. R. (2010). Hydrogen and oxygen stable isotope ratios of milk in the united states. *J. Agric. Food Chem.* 58, 2358–2363. doi: 10.1021/jf904151c
- Cilliers, J. (2017). "Fertility, growth and the future of aid in sub-Saharan Africa," in *Growth and the Future of Aid in Sub-Saharan Africa* (New York: Springer).
- Codron, D., Codron, J., Lee-Thorp, J. A., Sponheimer, M., Ruitter, D., and Brink, J. S. (2007). Stable isotope characterization of mammalian predator-prey relationships in a South African savanna. *Eur. J. Wildl. Res.* 3, 161–170. doi: 10.1007/s10344-006-0075-x
- Craig, H. (1961). Isotopic variations in meteoric waters. *Science* 133, 1702–1703. doi: 10.1126/science.133.3465.1702
- Crosier, A., Byron, M., and Comizzoli, P. (2022). Connecting the spots: understanding cheetah reproduction to improve assisted breeding and population management. *Theriogenology* 185, 70–77. doi: 10.1016/j.theriogenology.2022.03.025
- Crosier, A. E., Wachter, B., Schulman, M., Luders, I., Koester, D., Wielebnowski, N., et al. (2018). "Reproductive physiology of the cheetah and assisted reproductive techniques," in *Cheetahs: Biology and Conservation*; ed A. Valutkevich (London: Elsevier Inc.), 385–402.
- Dalberg, W. (2012). *Fighting Illicit Wildlife Trafficking*. Gland: WWF International.
- Daut, E. F., Brightsmith, D. J., and Peterson, M. J. (2015). Role of non-governmental organizations in combating illegal wildlife-pet trade in peru. *J. Nat. Conservat.* 24, 72–82. doi: 10.1016/j.jnc.2014.10.005
- De Luca, A., Boisseau, N., Tea, I., Louvet, I., Robins, R. J., Forhan, A., et al. (2012).  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in hair from newborn infants and their mothers: a cohort study. *Pediatr. Res.* 71, 598–604. doi: 10.1038/pr.2012.3
- De Waal, H., Osthoff, G., Hugo, A., Myburgh, J., and Botes, P. (2004). The composition of African lion (*Panthera leo*) milk collected a few days postpartum. *Mammalian Biol.* 69, 375–383. doi: 10.1078/1616-5047-00159
- Derocher, A. E., Andriashek, D., and Arnould, J. P. (1993). Aspects of milk composition and lactation in polar bears. *Can. J. Zool.* 71, 561–567. doi: 10.1139/z93-077
- Di Minin, E., Slotow, R., Hunter, L. T., Pouzols, F. M., Toivonen, T., Verburg, P. H., et al. (2016). Global priorities for national carnivore conservation under land use change. *Sci. Rep.* 6, 23814. doi: 10.1038/srep23814
- Durant, S. M., Mitchell, N., Groom, R., Pettorelli, N., Ipavec, A., Jacobson, A. P., et al. (2017). The global decline of cheetah *Acinonyx jubatus* and what it means for conservation. *Proc. Natl. Acad. Sci. U.S.A.* 114, 528–533. doi: 10.1073/pnas.1611122114
- Eaton, R. L. (1970). Hunting behavior of the cheetah. *J. Wildlife Manag.* 34, 56–67. doi: 10.2307/3799492
- Farhadinia, M. (2004). The last stronghold: cheetah in Iran. *Cat News* 40, 11–14.
- Gehre, M., Renpenning, J., Gilevska, T., Qi, H., Coplen, T. B., Meijer, H. A., et al. (2015). On-line hydrogen-isotope measurements of organic samples using elemental chromium: an extension for high temperature elemental-analyzer techniques. *Anal. Chem.* 87, 5198–5205. doi: 10.1021/acs.analchem.5b00085
- Hatch, K. A., Crawford, M. A., Kunz, A. W., Thomsen, S. R., Eggett, D. L., Nelson, S. T., et al. (2006). An objective means of diagnosing anorexia nervosa and bulimia nervosa using  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios in hair. *Rapid Commun. Mass Spectrom.* 20, 3367–3373. doi: 10.1002/rcm.2740
- Hijmans, R. J., and Van Etten, J. (2015). Raster: geographic data analysis and modeling. *R package version 2.3–40*.
- Hobson, K., Van Wilgenburg, S., Wassenaar, L., Powell, R., Still, C., and Craine, J. (2012). A multi-isotope ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ ) feather isoscape to assign afro-tropical migrant birds to origins. *Ecosphere* 3, 1–20. doi: 10.1890/ES12-00018.1
- Hobson, K. A., Alisauskas, R. T., and Clark, R. G. (1993). Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: Implications for isotopic analyses of diet. *Condor* 95, 388–394. doi: 10.2307/1369361
- Hobson, K. A., Atwell, L., and Wassenaar, L. I. (1999). Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proc. Natl. Acad. Sci. U.S.A.* 96, 8003–8006. doi: 10.1073/pnas.96.14.8003
- Hobson, K. A., and Koehler, G. (2015). On the use of stable oxygen isotope ( $\delta^{18}\text{O}$ ) measurements for tracking avian movements in North America. *Ecol. Evol.* 5, 799–806. doi: 10.1002/ece3.1383
- Hobson, K. A., and Wassenaar, L. I. (2008). *Tracking Animal Migration With Stable Isotopes*. Academic Press. doi: 10.1016/S1936-7961(07)00006-1
- Hobson, K. A., and Wassenaar, L. I. (2018). *Tracking Animal Migration With Stable Isotopes*. Academic Press.
- Hobson, K. A., Wunder, M. B., Van Wilgenburg, S. L., Clark, R. G., and Wassenaar, L. I. (2009). A method for investigating population declines of migratory birds using stable isotopes: origins of harvested lesser scaup in North America. *PLoS ONE* 4, e7915. doi: 10.1371/journal.pone.0007915
- Jenkins, S. G., Partridge, S. T., Stephenson, T. R., Farley, S. D., and Robbins, C. T. (2001). Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. *Oecologia* 129, 336–341. doi: 10.1007/s004420100755
- Koehler, G., and Hobson, K. A. (2019). Tracking cats revisited: placing terrestrial mammalian carnivores on  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  isoscapes. *PLoS ONE* 14, e0221876. doi: 10.1371/journal.pone.0221876
- Koehler, G., Kardynal, K. J., and Hobson, K. A. (2019). Geographical assignment of polar bears using multi-element isoscapes. *Sci. Rep.* 9, 9390. doi: 10.1038/s41598-019-45874-w
- Kornel, B. E., Werner, T., Roßmann, A., and Schmidt, H.-L. (1997). Measurement of stable isotope abundances in milk and milk ingredients—a possible tool for origin assignment and quality control. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A* 205, 19–24. doi: 10.1007/s002170050117
- Levin, N. E., Zipser, E. J., and Cerling, T. E. (2009). Isotopic composition of waters from ethiopia and kenya: Insights into moisture sources for eastern africa. *J. Geophys. Res. Atmospheres* 114, D23. doi: 10.1029/2009JD012166
- Magozzi, S., Vander Zanden, H., Wunder, M., and Bowen, G. (2019). Mechanistic model predicts tissue-environment relationships and trophic shifts in animal hydrogen and oxygen isotope ratios. *Oecologia* 191, 777. doi: 10.1007/s00442-019-04532-8
- Marker, L. (2019). Loving a species to death. *Biodiversity* 20, 50–55. doi: 10.1080/14888386.2019.1591300

- Marker, L. L., Muntifering, J., Dickman, A., Mills, M., and Macdonald, D. (2003). Quantifying prey preferences of free-ranging namibian cheetahs. *South Afr. J. Wildlife Res.* 33, 43–53.
- Marker-Kraus, L. (1997). History of the Cheetah: *Acinonyx jubatus* in zoos 1829–1994. *Int. Zoo Yearbook* 35, 27–43. doi: 10.1111/j.1748-1090.1997.tb01186.x
- McKechnie, A. E., Wolf, B. O., and Del Rio, C. M. (2004). Deuterium stable isotope ratios as tracers of water resource use: an experimental test with rock doves. *Oecologia* 140, 191–200. doi: 10.1007/s00442-004-1564-9
- Meier-Augenstein, W., Hobson, K. A., and Wassenaar, L. I. (2013). Critique: measuring hydrogen stable isotope abundance of proteins to infer origins of wildlife, food and people. *Bioanalysis* 5, 751–767. doi: 10.4155/bio.13.36
- Mekota, A.-M., Grupe, G., Ufer, S., and Cuntz, U. (2006). Serial analysis of stable nitrogen and carbon isotopes in hair: monitoring starvation and recovery phases of patients suffering from anorexia nervosa. *Rapid Commun. Mass Spectrom.* 20, 1604–1610. doi: 10.1002/rcm.2477
- Melzer, E., and Schmidt, H. (1987). Carbon isotope effects on the pyruvate dehydrogenase reaction and their importance for relative carbon-13 depletion in lipids. *J. Biol. Chem.* 262, 8159–8164. doi: 10.1016/S0021-9258(18)47543-6
- Mills, M. L. (1984). Prey selection and feeding habits of the large carnivores in the southern Kalahari. *Koedoe* 27, 281–294. doi: 10.4102/koedoe.v27i2.586
- Montanari, S., and Amato, G. (2015). Discrimination factors of carbon and nitrogen stable isotopes from diet to hair and scat in captive tigers (*Panthera tigris*) and snow leopards (*uncia uncia*). *Rapid Commun. Mass Spectrom.* 29, 1062–1068. doi: 10.1002/rcm.7194
- O'Brien, S. J., Roelke, M. E., Marker, L., Newman, A., Winkler, C., Meltzer, D., et al. (1985). Genetic basis for species vulnerability in the cheetah. *Science* 227, 1428–1434. doi: 10.1126/science.2983425
- O'Brien, S. J., Wildt, D. E., and Bush, M. (1986). The cheetah in genetic peril. *Sci. Am.* 254, 84–95. doi: 10.1038/scientificamerican0586-84
- Osthoff, G., Hugo, A., and De Wit, M. (2006). The composition of cheetah (*Acinonyx jubatus*) milk. *Comparat. Biochem. Physiol. B Biochem. Mol. Biol.* 145, 265–269. doi: 10.1016/j.cbpb.2006.05.016
- Parnig, E., Crumacker, A., and Kurle, C. M. (2014). Variation in the stable carbon and nitrogen isotope discrimination factors from diet to fur in four felid species held on different diets. *J. Mammal.* 95, 151–159. doi: 10.1644/13-MAMM-A-014.1
- Pietsch, S. J., Hobson, K. A., Wassenaar, L. I., and Tütken, T. (2011). Tracking cats: problems with placing feline carnivores on  $\delta^{18}\text{O}$ ,  $\delta\text{D}$  isoscapes. *PLoS ONE* 6, e24601. doi: 10.1371/journal.pone.0024601
- Pires, S. F. (2012). The illegal parrot trade: a literature review. *Global Crime* 13, 176–190. doi: 10.1080/17440572.2012.700180
- Pires, S. F., and Moretto, W. D. (2011). Preventing wildlife crimes: solutions that can overcome the 'tragedy of the commons'. *Eur. J. Crim. Policy Res.* 17, 101–123. doi: 10.1007/s10610-011-9141-3
- Polischuk, S. C., Hobson, K. A., and Ramsay, M. A. (2001). Use of stable-carbon and nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. *Can. J. Zool.* 79, 499–511. doi: 10.1139/z01-007
- Qi, H., Coplen, T. B., and Wassenaar, L. I. (2011). Improved online  $\delta^{18}\text{O}$  measurements of nitrogen- and sulfur-bearing organic materials and a proposed analytical protocol. *Rapid Commun. Mass Spectrom.* 25, 2049–2058. doi: 10.1002/rcm.5088
- R Core Team (2021). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Ripple, W. J., Estes, J. A., Beschta, R. L., Wilmers, C. C., Ritchie, E. G., Hebblewhite, M., et al. (2014). Status and ecological effects of the world's largest carnivores. *Science* 343, 1241484. doi: 10.1126/science.1241484
- Sachse, D., Billault, I., Bowen, G. J., Chikaraishi, Y., Dawson, T. E., Feakins, S. J., et al. (2012). Molecular paleohydrology: interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. *Annu. Rev. Earth Planet. Sci.* 40, 221–249. doi: 10.1146/annurev-earth-042711-105535
- Schoeller, D. A., Leitch, C. A., and Brown, C. (1986). Doubly labeled water method: in vivo oxygen and hydrogen isotope fractionation. *Am. J. Physiol. Regulat. Integr. Compar. Physiol.* 251, R1137–R1143. doi: 10.1152/ajpregu.1986.251.6.R1137
- Sessions, A. L., Burgoyne, T. W., Schimmelmann, A., and Hayes, J. M. (1999). Fractionation of hydrogen isotopes in lipid biosynthesis. *Org. Geochem.* 30, 1193–1200. doi: 10.1016/S0146-6380(99)00094-7
- Soto, D. X., Koehler, G., Wassenaar, L. I., and Hobson, K. A. (2017). Re-evaluation of the hydrogen stable isotopic composition of keratin calibration standards for wildlife and forensic science applications. *Rapid Commun. Mass Spectrom.* 31, 1193–1203. doi: 10.1002/rcm.7893
- Soto, D. X., Wassenaar, L. I., Hobson, K. A., and Catalan, J. (2011). Effects of size and diet on stable hydrogen isotope values ( $\delta\text{D}$ ) in fish: implications for tracing origins of individuals and their food sources. *Can. J. Fish. Aquat. Sci.* 68, 2011–2019. doi: 10.1139/f2011-112
- Spinage, C., Ryan, C., and Shedd, M. (1980). Food selection by the Grant's gazelle. *Afr. J. Ecol.* 18, 19–25. doi: 10.1111/j.1365-2028.1980.tb00267.x
- Still, C. J., and Powell, R. L. (2010). "Continental-scale distributions of vegetation stable carbon isotope ratios," in *Isoscapes*, eds J. West, G. Bowen, T. Dawson, and K. Tu (Dordrecht: Springer). doi: 10.1007/978-90-481-3354-3\_9
- Storm-Suke, A., Wassenaar, L. I., Nol, E., and Norris, D. R. (2012). The influence of metabolic rate on the contribution of stable-hydrogen and oxygen isotopes in drinking water to quail blood plasma and feathers. *Funct. Ecol.* 26, 1111–1119. doi: 10.1111/j.1365-2435.2012.02014.x
- Terzer, S., Wassenaar, L., Araguás-Araguás, L., and Aggarwal, P. (2013). Global isoscapes for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in precipitation: improved prediction using regionalized climatic regression models. *Hydrol. Earth Syst. Sci.* 17, 4713. doi: 10.5194/hess-17-4713-2013
- Terzer-Wassmuth, S., Wassenaar, L. I., Welker, J. M., and Araguás-Araguás, L. J. (2021). Improved high-resolution global and regionalized isoscapes of  $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$  and d-excess in precipitation. *Hydrol. Process.* 35, e14254. doi: 10.1002/hyp.14254
- Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., and Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implications for  $\delta^{13}\text{C}$  analysis of diet. *Oecologia* 57, 32–37. doi: 10.1007/BF00379558
- Tieszen, L. L., Hein, D., Qvortrup, S., Troughton, J., and Imbamba, S. (1979a). Use of  $\delta^{13}\text{C}$  values to determine vegetation selectivity in East African herbivores. *Oecologia* 37, 351. doi: 10.1007/BF00347911
- Tieszen, L. L., Senyimba, M. M., Imbamba, S. K., and Troughton, J. H. (1979b). The Distribution of C3 and C4 grasses and carbon isotope discrimination along an altitudinal and moisture gradient in Kenya. *Oecologia* 37, 337–350. doi: 10.1007/BF00347910
- Topalov, K., Schimmelmann, A., Polly, P. D., Sauer, P. E., and Lowry, M. (2013). Environmental, trophic, and ecological factors influencing bone collagen  $\delta^2\text{H}$ . *Geochim. Cosmochim. Acta* 111, 88–104. doi: 10.1016/j.gca.2012.11.017
- Tordiffe, A. S., Wachter, B., Heinrich, S. K., Reyers, F., and Mienie, L. J. (2016). Comparative serum fatty acid profiles of captive and free-ranging cheetahs (*Acinonyx jubatus*) in Namibia. *PLoS ONE* 11, e0167608. doi: 10.1371/journal.pone.0167608
- Tricorache, P., Nowell, K., Wirth, G., Mitchell, N., Boast, L. K., and Marker, L. (2018). "Pets and pelts: Understanding and combating poaching and trafficking in cheetahs," in *Biodiversity of the World-Cheetahs: Biology and Conservation*, 1st Edn (San Diego, CA: Elsevier), 191–205.
- Van Wilgenburg, S. L., and Hobson, K. A. (2011). Combining stable-isotope ( $\delta\text{D}$ ) and band recovery data to improve probabilistic assignment of migratory birds to origin. *Ecol. Appl.* 21, 1340–1351. doi: 10.1890/09-2047.1
- Vander Zanden, H. B., Nelson, D. M., Wunder, M. B., Conkling, T. J., and Katzner, T. (2018). Application of isoscapes to determine geographic origin of terrestrial wildlife for conservation and management. *Biol. Conserv.* 228, 268–280. doi: 10.1016/j.biocon.2018.10.019
- Vander Zanden, H. B., Soto, D. X., Bowen, G. J., and Hobson, K. A. (2016). Expanding the isotopic toolbox: applications of hydrogen and oxygen stable isotope ratios to food web studies. *Front. Ecol. Evol.* 4, 20. doi: 10.3389/fevo.2016.00020
- Velho, N., Karanth, K. K., and Laurance, W. F. (2012). Hunting: a serious and understudied threat in India, a globally significant conservation region. *Biol. Conserv.* 148, 210–215. doi: 10.1016/j.biocon.2012.01.022
- Voigt, C. C., Thalwitzer, S., Melzheimer, J., Blanc, A.-S., Jago, M., and Wachter, B. (2014). The conflict between cheetahs and humans on Namibian farmland elucidated by stable isotope diet analysis. *PLoS ONE* 9, e01917. doi: 10.1371/journal.pone.01917
- Warchol, G. L. (2004). The transnational illegal wildlife trade. *Crim. Justice Stud.* 17, 57–73. doi: 10.1080/08884310420001679334
- Warchol, G. L., Zupan, L. L., and Clack, W. (2003). Transnational criminality: an analysis of the illegal wildlife market in Southern Africa. *Int. Crim. Justice Rev.* 13, 1–27. doi: 10.1177/105756770301300101
- West, J. B., Bowen, G. J., Dawson, T. E., and Tu, K. P. (2009). *Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping*. Dordrecht: Springer. doi: 10.1007/978-90-481-3354-3
- Wilson, G., Mackenzie, D., Brookes, I., and Lyon, G. (1988). Importance of body tissues as sources of nutrients for milk synthesis in the cow, using  $^{13}\text{C}$  as a marker. *Br. J. Nutr.* 60, 605–617. doi: 10.1079/BJN19880131
- Wolf, C., and Ripple, W. J. (2017). Range contractions of the world's large carnivores. *R. Soc. Open Sci.* 4, 170052. doi: 10.1098/rsos.170052
- Wolf, N., Newsome, S. D., Peters, J., and Fogel, M. L. (2015). Variability in the routing of dietary proteins and lipids to consumer tissues influences tissue-specific isotopic discrimination. *Rapid Commun. Mass Spectrom.* 29, 1448–1456. doi: 10.1002/rcm.7239