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Variation in the stable carbon and nitrogen isotope discrimination factors from diet to fur in four felid species held on different diets

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Stable carbon ($^{13}C/^{12}C$; $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$; $\delta^{15}N$) isotope ratios are best used to assess wild animal diets when the isotopic differences between consumers and diets are known. These differences are called discrimination factors (expressed with Δ notation). We report the 1st $\Delta^{13}C$ and $\Delta^{15}N$ values between diet and fur from captive individuals held on controlled diets for 7 months and representing 4 felid species: African lions (*Panthera leo*), bobcats (*Lynx rufus*), Canada lynx (*Lynx canadensis*), and mountain lions (*Puma concolor*). All animals were fed a mix of diet items (beef, beef rib, a commercial carnivore diet, chicken, mice, rats, turkey, and turkey wings) that was consistent throughout their molting period. Weekly diet composition was determined by the percentage of mass of each diet item and overall $\delta^{13}C$ and $\delta^{15}N$ values were calculated for each animal's diet. The mean $\Delta^{13}C$ and $\Delta^{15}N$ values ($\pm$ *SD*) between felid fur and their non–lipid-extracted diets were 1.1‰ $\pm$ 0.2‰ and 3.5‰ $\pm$ 0.0‰, respectively (African lion, $n = 1$ animal sampled at 2 intervals); 5.5‰ $\pm$ 0.5‰ and 4.1‰ $\pm$ 0.1‰, respectively (bobcats, $n = 3$); 2.4‰ and 3.3‰, respectively (Canada lynx, $n = 1$); and 4.7‰ $\pm$ 0.6‰ and 4.5‰ $\pm$ 0.2‰, respectively (mountain lions, $n = 2$). Variations in $\Delta^{13}C$ and $\Delta^{15}N$ values among species were likely due to dietary differences and we recommend the use of the $\Delta^{13}C$ (5.5 $\pm$ 0.5) and $\Delta^{15}N$ (4.1 $\pm$ 0.1) values obtained from the bobcats for future determinations of wild felid foraging ecology as they were held on diets composed of 100% whole animals and animal parts, which best reflects diets of wild felids.

Key words: African lion, bobcat, Canada lynx, captive feeding study, mountain lion, stable isotope analysis

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Conservation and population management of large predators requires an understanding of their foraging ecology and habitat use, and the management and monitoring of their prey populations (Chapron et al. 2008; Palomares et al. 2010). However, the intensive time and monetary commitment required to obtain this type of information from elusive, wide-ranging mammals such as felids means that these data are frequently lacking. When instrument tracking or other constant monitoring is not possible, indirect methods such as stable isotope analysis of found predator tissue can be a more efficient and practical method by which to collect these data.

Natural variations in stable isotope ratios of carbon ($^{13}C/^{12}C$, reported as $\delta^{13}C$ values) and nitrogen ($^{15}N/^{14}N$, reported as $\delta^{15}N$ values) from animal tissues provide information on predator–prey relationships and can be used to make inferences about an individual's foraging ecology and habitat use (Ben-David and Flaherty 2012). Stable isotope ratios vary between animal tissues and their diets because of isotope fractionation and routing and this difference is called the trophic discrimination factor (Martínez del Rio et al. 2009). It is reported as $\Delta^{13}C$ for carbon and $\Delta^{15}N$ for nitrogen, which is defined as $\Delta^{13}C_{tissue–diet} = \delta^{13}C_{tissue} - \delta^{13}C_{diet}$ (and similarly for nitrogen).

Because $\delta^{13}C$ and $\delta^{15}N$ values vary with metabolic processes, these stable isotope ratios can differ among species and among individuals within the same species because of the type of tissue analyzed, nutritional stress, water stress, reproductive status, an individual's unique diet, and age (Adams and Sterner 2000; Roth and Hobson 2000; Robbins et al. 2010; Poupin et al. 2011). Given the high degree of variation, using assumed discrimination factors in stable isotope mixing models can result in substantial errors in the estimation of wild animal foraging ecology (Bond and Diamond 2011; Phillips 2012). Therefore, determination of

discrimination factors using captive animals held on controlled diets is important for accurate modeling of animal diets in the wild.

We determined the stable carbon and nitrogen isotope discrimination factors for fur from 4 species of captive felids: African lions (*Panthera leo*), bobcats (*Lynx rufus*), Canada lynx (*Lynx canadensis*), and mountain lions (*Puma concolor*) held on known diets for 7 months. The availability of fur from captive individuals held on a consistent diet in this study presented a unique opportunity to obtain isotope discrimination factors that can be used in future studies of wild felid foraging ecology. This is especially important for biological conservation because felids are top-level consumers and therefore responsible for shaping substantial components of their ecosystems via predation and trophic cascades (Krebs et al. 1995; Sinclair et al. 2003, 2010; Terborgh and Feeley 2010; Estes et al. 2011). In addition, expanding human development continues to encroach on the few remaining habitats spacious enough to support large terrestrial predators (Hunter et al. 2007) and, according to the United States Endangered Species Act and the *IUCN Red List*, the felids in this study range from endangered (certain subspecies of *P. concolor*) to threatened (*L. canadensis* and other subspecies of *P. concolor*), to vulnerable (*P. leo*), to stable (*L. rufus*), further making their conservation a priority.

The African lion is the only felid in this study not native to North America. African lions are found mostly in eastern and southern Africa, but fragmented populations also exist in western and central Africa. A pride of lions can occupy a home range for many generations, holding the territory until the resident male is defeated. Large prides usually defend territories ranging from 15 to 219 km^2 with a mean area of 56 km^2 that contain areas rich in resources (Bauer and Merwe 2004; Mosser and Packer 2009). Lions are opportunistic hunters that usually prey on medium to large mammals such as warthogs, African buffalo, seals, rhinoceros, elephants, and humans (Bridgeford 1985; Hayward and Kerley 2005).

Bobcats have the widest habitat distribution in North America of any native cat (Delibes et al. 1997). They generally inhabit arid areas in the northwest, northern temperate forests, and some southeastern subtropical swamps (Larivière and Walton 1997). Bobcat population size and movements are closely tied with that of their prey (Larivière and Walton 1997) and the males occupy and protect large home ranges in order to maintain their polygynous social order (Chamberlain et al. 2003). Bobcats hunt a wide range of herbivores and, occasionally, other carnivores. Their diet generally consists of deer, squirrels, snowshoe hares (*Lepus americanus*), and a variety of rodents (Larivière and Walton 1997).

The Canada lynx is the most north-ranging wild felid of North America and are found in various habitats ranging from Canada and Alaska to the western, northeastern, and central northern United States (Parker et al. 1983; Koehler 1990). The diet of the Canada lynx is varied and includes grouse, squirrels, snowshoe hares, weasels, and deer (Squires and Ruggier 2010), but snowshoe hares account for 96% of the diet and populations of Canada lynx in the Yukon, Canada, demonstrate well-documented fluctuations that mirror the population of snowshoe hares (Krebs et al. 1995).

The mountain lion also is native to North America and occupies the largest geographic range of any terrestrial mammal in the Western Hemisphere (Sunquist and Sunquist 2002), spanning many habitats from Canada to southern South America (Grigione et al. 2002). Mountain lions prey mostly on deer, hares, and wild birds (Irarte et al. 1990; Rau and Jimenez 2002), but they also have been known to prey on domestic animals and humans. Their population structure is poorly understood and is further complicated by increasing habitat loss and fragmentation (Caso et al. 2008). Mountain lions play important ecological roles as an indicator species for habitat connectivity and loss and, as an umbrella species, are targeted for evaluation in setting conservation and management priorities (Beier 1993; Penrod 2001; Logan and Sweanor 2001; Ernest et al. 2003).

Diets of wild animals are generally determined by utilizing a variety of methods such as fecal analysis, fistulation, and stomach content analysis, but these methods only provide a picture of an animal's most recent meals and do not illustrate long-term feeding habits. These techniques also can be difficult to employ, especially on predators such as the felids in this study that are difficult to find, dangerous to work with, and primarily nocturnal. Stable isotope analysis of found tissues such as fur shed within a wild animal's habitat range is a noninvasive method of analyzing foraging ecology and habitat use. Stable isotope analysis can reconstruct an individual's feeding behavior over a longer time period than scat or stomach contents analyses (Kurle 2002, 2009) and can provide a useful tool for conservation biologists to better understand the diet and movement patterns of wild animals. To the best of our knowledge, this is the 1st study to obtain stable isotope discrimination factors for fur from 4 felid species and our results will be useful as a reference for future management and conservation efforts.

Materials and Methods

Fur and diet samples.—All animals in this study were held at The Fund for Animals Wildlife Center (hereafter, the Center) in Ramona, California. The Center houses permanent residents that cannot be released or transferred due to human imprinting or health issues, or both. We focused on 7 individuals that are referred to throughout the study by the names given to them by the Center: 1 African lion (Samson), 3 bobcats (Cleo, Pepper, and unknown), 1 Canada lynx (Linky), and 2 mountain lions (Tonka and Sheba). We collected fur samples opportunistically during daily feedings using a wooden backscratcher to comb fur from individuals. One clump of fur came from an unknown bobcat housed in 1 of the enclosures. The dates on which fur was collected varied due to limitations posed by opportunistic sampling.

All individuals were kept on strict diets following their own unique schedules (Table 1) from 1 January to 31 July 2011,

Table 1.—Weekly felid diet composition by percentage (%) of mass.

Animal	Beef	Beef rib[a]	CD[b]	Chicken[c]	Mice[d]	Rat[d]	Ground turkey	Turkey wing[e]	Total mass (kg)
African lion	0	0	87.5	0	0	0	12.5	0	14.5
Bobcat	0	10.5	0	0	36.8	42.2	0	10.5	4.3
Canada lynx	60.0	0	0	0	40.0	0	0	0	4.0
Mountain lion (male)	13.1	13.1	9.8	13.1	16.4	26.2	8.2	0	13.8
Mountain lion (female)	25.8	9.7	6.5	12.9	12.9	19.4	12.9	0	7.0

[a] No beef rib bones were ingested.

[b] Zoo Carnivore Diet 10 is a commercial carnivore diet with 10% fat. This was supplied by Natural Balance Pet Foods (Pacoima, California) and is a blend of beef, beef hearts, beet pulp, tricalcium phosphate, ground whole flaxseed, sodium chloride, choline chloride, taurine, vitamin E supplement, L-ascorbyl-2-polyphosphate (source of vitamin C), niacin, biotin, copper sulfate, vitamin A acetate, vitamin D3 supplement, menadione dimethyl-pyrimidinol bisulfite, riboflavin, pyridoxine hydrochloride, thiamin mononitrate, manganese sulfate, *d*-calcium pantothenate, folic acid, ethylenediamine dihydriodide, calcium iodate, and sodium selenite.

[c] Whole chickens were ingested, minus the feathers.

[d] Whole animals were ingested.

[e] Whole turkey wings were ingested, minus feathers.

and some took daily medication for chronic kidney disease (Samson [lion], Cleo [bobcat], and Sheba [mountain lion]), skeletal deformities and joint pain (Samson), or healthy coat and nails (Tonka [mountain lion]). In humans, stable isotopes in medication have been used to study metabolic disposition (Jacob et al. 1991). The medication administered to the individuals in that study did not significantly impact the individual's metabolism. Thus, it was assumed for this study that the medication taken by the subjects would not make a noticeable impact on the individual's stable isotope signatures.

The mice and rats used to feed the animals were supplied by RodentPro (Inglefield, Indiana) and the beef, chicken, and turkey were supplied by Seaport Meat Company (Spring Valley, California). We took subsamples of muscle at random from each diet item and we chose to analyze muscle samples rather than homogenized prey carcasses for 2 reasons: it was logistically impossible to fully homogenize whole prey items, and muscle tissue represents the bulk of digestible and assimilated material in the diet items (Therrien et al. 2011). We kept all prey samples frozen until sample preparation.

Stable isotope analysis.—Fur samples were agitated in petroleum ether to remove all potential contaminants, rinsed with deionized water, and dried in a 40°C oven for 24 h. We cut dry fur samples into 1- to 3-mm-long pieces. We freeze-dried all prey muscle samples for 12 h, then ground them to a powder by hand. Muscle samples were split into 2 components and one-half was lipid extracted following a method modified from Folch et al. (1957; Sweeting et al. 2006; Post et al. 2007). We placed ground samples in 15-ml glass centrifuge tubes, added 10 ml of petroleum ether (Dobush et al. 1985), capped the vials with perforated lids, and sonicated them for 10 min at 40 kHz in a 60°C water bath. We then centrifuged the tubes at 12,000 × *g* for 5 min, removed the petroleum ether with a pipette, rinsed each sample thoroughly with ultrapure water, and removed the excess water with a pipette. We added 10 ml of fresh ultrapure water to each tube, sonicated the tubes for 10 min at 40 kHz in a 60°C water bath, and centrifuged the sample at 12,000 × *g* for 5 min. We removed the excess water with a pipette and dried the samples in a drying oven at 40°C for 48 h. We rehomogenized the prey muscle samples and weighed ~0.7–1.0 mg of fur and muscle samples into 5 × 9-mm tin capsules and sent them to the Stable Isotope Laboratory at the Department of Earth and Marine Sciences at the University of California, Santa Cruz.

Stable carbon and nitrogen isotope ratios were analyzed using a CE1108 elemental analyzer (Carlo Erba, Milan, Italy) interfaced via a CONFLO III device to a Thermo-Electron Delta Plus XP mass spectrometer (Thermo-Finnigan, Bremen, Germany). We calculated δ values, where $\delta = (R_{sample}/R_{standard} - 1) \times 1{,}000$, and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The units are parts per thousand (per mil, ‰) deviations from the standard. We calculated the average precision for these data as the *SD* of the $\delta^{15}N$ and $\delta^{13}C$ values from a set of standards (acetanilide from A. Schimmelmann, Indiana University [see Schimmelmann et al. 2009]) and precision was 0.11‰ for nitrogen and 0.06‰ for carbon.

Data analysis.—All individuals were kept on a weekly diet schedule for which the weighted percentage of each diet item was calculated by comparing the total body mass per week of each diet item to the total body mass per week of all food consumed by each individual. Using this weighted percentage and the $\delta^{13}C$ and $\delta^{15}N$ values of each prey item and each felid's fur, we calculated the discrimination factors using the following equation:

$$\Delta X(‰) = (\delta X_{predator}) - \left[(\%_{prey\ 1} \times \delta X_{prey\ 1}) + (\%_{prey\ 2} \times \delta X_{prey\ 2}) + \ldots\right],$$

where ΔX(‰) is either the carbon or nitrogen isotope discrimination factor ($\Delta^{13}C$ or $\Delta^{15}N$), $\delta X_{predator}$ is the $\delta^{13}C$ or $\delta^{15}N$ value of the fur from each felid species, $\%_{prey}$ is the contribution of a specific prey item to the total diet (Table 1), and δX_{prey} is the $\delta^{13}C$ or $\delta^{15}N$ value of that particular prey item (Kurle 2002; Table 2).

All statistical tests were performed with Systat 13 (Systat Software, Inc. 2009) using parametric methods because all data met the assumptions for parametric tests. Values reported are means ± *SD* and significance was tested at the $\alpha = 0.05$ level.

Results

The $\delta^{13}C$ values of the prey samples were significantly higher after extracting the lipids (paired *t*-test, $P = 0.04$; Table

Table 2.—Stable carbon (C) and nitrogen (N) isotope values from lipid-extracted (LE) and non–lipid-extracted (NLE) diet items reported in parts per thousand (‰). Zoo Carnivore Diet 10 is a commercial carnivore diet (see details in Table 1).

	NLE $\delta^{13}C$	LE $\delta^{13}C$	NLE $\delta^{15}N$	LE $\delta^{15}N$	NLE % C	LE % C	NLE % N	LE % N	NLE C:N	LE C:N
Beef	−12.3	−12.3	5.5	6.1	34.4	66.4	10.2	20.3	3.4	3.3
Beef rib	−18.4	−18.1	6.1	6.4	82.8	35.4	17	8.6	4.9	4.1
CD	−20.2	−18.6	6.3	7.2	40.3	38.6	7.2	9.0	5.6	4.3
Chicken	−16.8	−17.1	2.9	2.9	78.2	55.6	8.4	16.6	9.4	3.4
Mice	−19.0	−18.8	5.7	6.0	46.1	46.5	10.9	13.2	4.2	3.5
Rat	−25.4	−24.7	2.5	3.0	60.4	43.9	17.7	13.3	3.4	3.3
Turkey	−20.1	−19.2	4.4	4.7	73.6	51.5	7.5	13.5	6.2	3.8
Turkey wing	−22.0	−20.4	3.1	2.4	44.7	68.5	4.7	21.5	9.5	3.2

2), and this was most pronounced for the prey items that were higher in lipids as indicated by their higher C:N ratios (carnivore diet, chicken, turkey, and turkey wing [Table 2]). This was expected because the synthesis of lipids discriminates against ^{13}C leading to lower $\delta^{13}C$ values in lipid-rich tissues (DeNiro and Epstein 1977). However, because the carbon in lipids contributes to the synthesis of amino acids in keratin, the key structural component of fur (O'Brien et al. 2002; Howland et al. 2003; Newsome et al. 2010), we are reporting the $\Delta^{13}C$ values between the felid fur and lipid-intact (non–lipid-extracted [NLE]) and lipid-extracted (LE) prey items (as per the recommendation in Newsome et al. [2010]).

The $\delta^{13}C$ values from the NLE animal diets were –20.2‰ (African lion), –21.9‰ (bobcat), –15.0‰ (lynx), –19.6‰ (male mountain lion), and –18.4‰ (female mountain lion) (Table 3). The $\delta^{13}C$ values from the LE animal diets were –18.7‰ (African lion), –21.4‰ (bobcat), –14.9‰ (lynx), –19.2‰ (male mountain lion), and –18.0‰ (female mountain lion [Table 3]). The mean $\delta^{13}C$ (± *SD*) values from the felid fur were –19.1‰ ± 0.2‰ (1 African lion sampled at 2 time intervals), –16.4‰ ± 0.5‰ (bobcat, $n = 3$), and –14.3‰ ± 0.3‰ (mountain lion, $n = 2$). The $\delta^{13}C$ value for the lynx was –12.6‰ (Table 3). The mean $\Delta^{13}C$ (± *SD*) values measured between felid fur and their NLE diets were 1.1‰ ± 0.2‰ (African lion), 5.5‰ ± 0.5‰ (bobcat), and 4.7‰ ± 0.6‰ (mountain lion). The $\Delta^{13}C$ value for the lynx was 2.4‰ (Table 3). The mean $\Delta^{13}C$ (± *SD*) values measured between felid fur and their LE diets were –0.4‰ ± 0.2‰ (African lion), 4.9‰ ± 0.5‰ (bobcat), and 4.3‰ ± 0.5‰ (mountain lion). The $\Delta^{13}C$ value for the lynx was 2.3‰ (Table 3).

We found no significant difference between the $\delta^{15}N$ values (paired *t*-test, $P = 0.13$) from LE and NLE prey muscle, but most prey items had somewhat higher $\delta^{15}N$ values after the lipid extraction (Table 2). This relationship has been observed in other studies that extract lipids using chloroform methanol as the solvent (Sweeting et al. 2006; Kojadinovic et al. 2008; Ruiz-Cooley et al. 2011), but is not expected when using petroleum ether (Dobush et al. 1985; Kurle et al. 2011) as we

Table 3.—Name, species, sex (female = F, male = M), age, body mass, sample collection date, stable isotope values (‰), discrimination factors (‰), and carbon to nitrogen (C:N) ratios for each individual and mean values (± *SD*) for each species group containing more than 1 data point. The $\Delta^{13}C$ values were calculated using the $\delta^{13}C$ values from prey tissues that were not lipid extracted (NLE) and lipid extracted (LE), whereas the $\Delta^{15}N$ values were calculated using the $\delta^{15}N$ values from prey tissues that were not lipid extracted (NLE). NA = not available.

						Animal fur		Diet			Discrimination factors			Animal fur
Individual	Species	Sex	Age (years)	Body mass (kg)	Sample	$\delta^{13}C$	$\delta^{15}N$	NLE $\delta^{13}C$	LE $\delta^{13}C$	NLE $\delta^{15}N$	NLE $\Delta^{13}C$	LE $\Delta^{13}C$	NLE $\Delta^{15}N$	C:N
Samson	African lion	M	10	159	23 May	−18.9	9.6	−20.2	−18.7	6.1	1.3	−0.2	3.5	3.0
Samson	African lion	M	10	159	7 September	−19.2	9.6	−20.2	−18.7	6.1	1.0	−0.5	3.5	2.9
Cleo	Bobcat	F	12	12	21 September	−16.3	8.3	−21.9	−21.4	4.1	5.6	5.1	4.2	3.0
Pepper	Bobcat	F	14	13	21 September	−16.0	8.2	−21.9	−21.4	4.1	5.9	5.4	4.1	3.1
Unknown[a]	Bobcat	NA	NA	NA	NA	−17.0	8.2	−21.9	−21.4	4.1	4.9	4.4	4.1	3.0
Linky	Canada lynx	M	14	14	16 May	−12.6	8.9	−15.0	−14.9	5.6	2.4	2.3	3.3	3.0
Tonka	Mountain lion	M	8	77	30 May	−14.5	9.1	−19.6	−19.2	4.5	5.1	4.7	4.6	2.9
Sheba	Mountain lion	F	14	50	10 June	−14.1	9.0	−18.4	−18.0	4.6	4.3	3.9	4.4	2.9
$\bar{X}$	African lion					−19.1 ± 0.2	9.6 ± 0.0	—	—	—	1.1 ± 0.2	−0.4 ± 0.2	3.5 ± 0.0	2.9 ± 0.0
	Bobcat					−16.4 ± 0.5	8.2 ± 0.1	—	—	—	5.5 ± 0.5	4.9 ± 0.5	4.1 ± 0.1	3.0 ± 0.0
	Mountain lion					−14.3 ± 0.3	9.1 ± 0.1	—	—	—	4.7 ± 0.6	4.3 ± 0.5	4.5 ± 0.2	2.9 ± 0.0

[a] Individual from which fur originated is unknown.

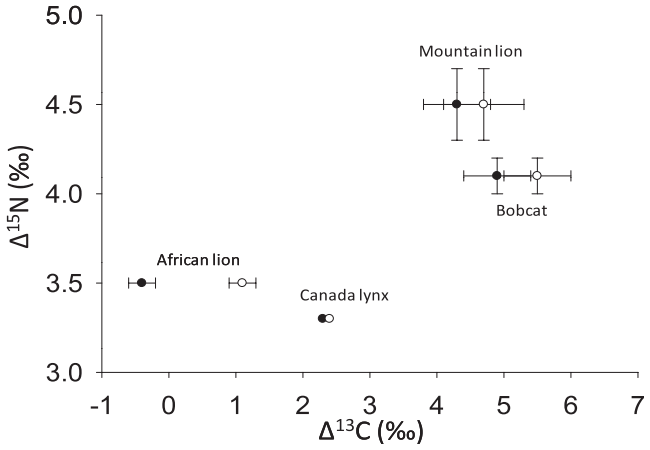

FIG. 1.—Mean (± *SD*) stable nitrogen and carbon isotope discrimination factors between diet and fur from captive felids. Open circles indicate $\Delta^{13}C$ values calculated from non–lipid-extracted (NLE) prey and closed circles indicate $\Delta^{13}C$ values calculated from lipid-extracted (LE) prey. All $\Delta^{15}N$ values were calculated using NLE prey.

did in this study. Because of these small differences, we calculated the $\Delta^{15}N$ values by comparing the $\delta^{15}N$ values from the felid fur with those from the NLE prey muscle samples.

The $\delta^{15}N$ values from the NLE animal diets were 6.1‰ (African lion), 4.1‰ (bobcat), 5.6‰ (lynx), 4.5‰ (male mountain lion), and 4.6‰ (female mountain lion [Table 2]). The mean $\delta^{15}N$ (± *SD*) values from the felid fur were 9.6‰ ± 0.0‰ (1 African lion sampled at 2 time intervals), 8.2‰ ± 0.1‰ (bobcat, $n = 3$), and 9.1‰ ± 0.1‰ (mountain lion, $n = 2$). The $\delta^{15}N$ value for the lynx was 8.9‰ (Table 3). The mean $\Delta^{15}N$ values (± *SD*) measured between felid fur and their diets were 3.5‰ ± 0.0‰ (African lion), 4.1‰ ± 0.1‰ (bobcat), and 4.5‰ ± 0.2‰ (mountain lion). The $\Delta^{15}N$ value for the lynx was 3.3‰ (Table 3).

The $\Delta^{13}C$ values from animals ordered lowest to highest were lion, lynx, mountain lion, and bobcat (Fig. 1). The $\Delta^{15}N$ values ordered lowest to highest were lynx, lion, bobcats, and mountain lions (Fig. 1). We were able to statistically compare mean $\Delta^{13}C$ and $\Delta^{15}N$ values between the mountain lions and bobcats; mountain lions had higher $\Delta^{15}N$ values, but the $\Delta^{13}C$ values were not different between species (*t*-tests; $\Delta^{15}N$, $t_3 = -4.49$, $P = 0.02$; $\Delta^{13}C$ for NLE prey, $t_3 = 3.18$, $P = 0.2$; $\Delta^{13}C$ for LE prey, $t_3 = 1.31$, $P = 0.3$; Table 3; Fig. 1).

DISCUSSION

Variation in $\Delta^{13}C$ and $\Delta^{15}N$ values among felids in this study.— Our study provides the 1st diet–tissue discrimination factors reported for any species of large felids. Therefore, we have no felid discrimination factors to compare with those found in this study. It is common to observe variation in $\Delta^{13}C$ and $\Delta^{15}N$ values among tissues within the same species and among the same tissues from the same or similar species held on different diets (Martínez del Rio et al. 2009). We also observed significant differences among species. The $\Delta^{15}N$ values from the mountain lions were greater than those from the bobcats, and the $\Delta^{13}C$ values from the bobcats were the highest, but they were not significantly different than those from the mountain lions. Although we were unable to test for significant differences because of small sample size, the lion and lynx had the lowest $\Delta^{13}C$ and $\Delta^{15}N$ values of all 4 species. It is important to note that the range of $\Delta^{15}N$ values was small (3.3–4.6‰) and represents less than 1 trophic level, whereas the range of $\Delta^{13}C$ values was quite large (–0.5–5.9‰). In addition, the $\Delta^{15}N$ values we observed fall within those observed for various tissues from other mammalian carnivores (e.g., Hobson et al. 1996; Kurle 2002; Newsome et al. 2010; Ben-David et al. 2012), whereas the $\Delta^{13}C$ values we observed were somewhat higher.

The variations in $\Delta^{13}C$ and $\Delta^{15}N$ values we observed among species are likely due to the different diets consumed by the felids in our study. The presence of lipids within tissues can significantly lower their $\delta^{13}C$ values (Post et al. 2007), leading many stable isotope practitioners to extract lipids so as to better compare isotope values among prey and consumers with different lipid contents. However, components from dietary lipids can be routed to keratin, the structural proteins in fur, during keratin synthesis (Newsome et al. 2010) and therefore affect the $\delta^{13}C$ and subsequent $\Delta^{13}C$ values of consumer fur. The prey items in this study varied in lipid content as demonstrated by their C:N ratios, which ranged from 3.4 to 9.4, indicating a percent lipid content from near zero to more than 30% (Post et al. 2007; Table 1). Animals ingesting prey with a higher lipid content will have more lipid-derived macromolecules allocated to their fur than animals ingesting lower-lipid prey items, and subsequently their fur will have lower $\delta^{13}C$ values. Therefore, the measurement of their $\Delta^{13}C$ values using LE prey items will lead to artificially low $\Delta^{13}C$ values and a misrepresentation of the animals' diet (Newsome et al. 2010).

We observed this relationship with the $\Delta^{13}C$ values from the lion, which ingested the commercial carnivore mix and ground turkey, both of which were higher in lipids as indicated by their C:N ratios (5.6 and 6.2, respectively or ~30% lipid—Post et al. 2007). The $\Delta^{13}C$ values between fur and the diet items that were lipid extracted were nearly zero (artificially low), whereas they were 1.1‰ when calculated with the lipid-intact prey (Table 3). Newsome et al. (2010) demonstrated this same pattern with vibrissae from sea otters that consumed differing amounts of lipid via their variable ingestion of high-lipid sea urchins. As mentioned, most stable isotope practitioners remove lipids from prey and predator tissues to eliminate the bias associated with their low $\delta^{13}C$ values. However, for the most accurate estimations of $\Delta^{13}C$ values, Newsome et al. (2010) recommend keeping lipids intact for prey items when calculating $\Delta^{13}C$ values for animals ingesting high proportions of lipid-rich prey. We agree with this recommendation for most of the animals in this study. The lynx is the only animal maintained exclusively on low-lipid diet items, beef and mice, as indicated by their low C:N ratios (3.4 and 4.2, respectively), and lipid extraction of these diet items showed no measureable difference in the lynx's $\Delta^{13}C$ values.

In addition to variation in $\Delta^{13}C$ values caused by differential routing of lipid macromolecules from lipid-rich versus low-lipid diet items, differences in the relative proportions of the amino acids within and incorporated from the different diets into the felid fur could be driving the observed $\Delta^{13}C$ differences between species. This is because amino acids have widely varying $\delta^{13}C$ values, depending upon their source (DeNiro and Epstein 1978; O'Brien et al. 2005; Larsen et al. 2009; Martínez del Rio et al. 2009). Animals make tissues using amino acids whose carbon skeletons are conserved from dietary sources to varying degrees. The carbon skeletons making up essential amino acids must be ingested, whereas the carbon skeletons in nonessential amino acids can come from the diet or be assembled endogenously from parts of other macromolecules (DeNiro and Epstein 1978; O'Brien et al. 2002; Bequette 2003; Martínez del Rio et al. 2009). As mentioned, the sources of amino acids available to the felids in our study varied by diet and ranged from whole animals (chicken, mice, and rats), to animal parts (beef, beef ribs, ground turkey, and turkey wings), to a commercial diet containing plant (beet and flaxseed) and animal (beef and beef hearts) sources and supplemental taurine. The differential $\delta^{13}C$ values in these varying sources of dietary amino acids are likely contributing to the differences observed in the $\Delta^{13}C$ values.

Finally, the $\Delta^{13}C$ values from the felid species in this study were as high as 5.9‰, which are considerably higher than the generally assumed 0–1.0‰ (Kelly 2000; Clementz and Koch 2001). Keratinous tissues typically have higher $\delta^{13}C$ values (and thus higher $\Delta^{13}C$ values) than more metabolically active tissues such as muscle, blood, and liver (DeNiro and Epstein 1978; Tieszen et al. 1983; Hobson and Clark 1992; Hobson et al. 1996; Kelly 2000; Kurle 2009; Federer et al. 2010; Lecomte et al. 2011). This is likely because of the distinct amino acid makeup of α-keratin, the structural component of fur and whiskers (Newsome et al. 2010; Marshall et al. 1991), and the factor that is likely contributing to the fairly high $\Delta^{13}C$ values we observed in felid fur.

Dietary differences also can cause variation in $\Delta^{15}N$ values in several ways. Previous research has demonstrated that animals held on high–protein-quality diets typically have lower $\Delta^{15}N$ values (Robbins et al. 2010) because the process of nitrogen discrimination, or preferentially retaining ^{15}N during nitrogen metabolism and excretion, decreases with an increase in protein quality (Roth and Hobson 2000). Protein quality is measured by the degree to which the amino acid composition of the diet matches the amino acid requirements of the consumer. If the dietary amino acids are supplied in roughly the same relative abundance as in consumer tissues, and are supplied at a rate that meets daily needs, then the diet has high protein quality. Alternatively, even a diet with high percent protein and percent nitrogen, and a low C:N ratio, may be poor quality if it is low in critical essential amino acids. In such a situation, the animal will need to consume more food to meet the daily requirement of the essential amino acid(s), and will likely catabolize "extra" amino acid R-groups for energy or storage, and shed excess NH_4 as waste.

We did not measure the specific amino acid requirements of the different felid species, nor do we know the amino acid contents of the different diet items. The meat-rich diets of carnivores contain all required amino acids (Morris 1985), so we are confident that all animals were receiving diets of adequate quality. However, either the beef and beef hearts or the plant material (beets and flaxseeds), or both, that provide the protein in the commercial carnivore diet may provide a more complete amino acid profile for the lion compared to the various animal tissues provided to the other species, thereby contributing to the lion's lower $\Delta^{15}N$ values. In addition, the lynx is the only species that receives a high percentage of its diet in the form of beef (60.0% versus 13.1–25.8% for the mountain lions), and its $\Delta^{15}N$ value was nearly identical to that of the lion. Perhaps the beef provides a higher quality protein for these felids than the mixture of other diet items that compose the bulk of their diets.

Additionally, animals at steady state ingesting higher quantities of protein, as measured by percent nitrogen or percent protein, have higher $\Delta^{15}N$ values than animals ingesting less protein (Koch 2007). Carnivores on a high-protein diet would have higher $\Delta^{15}N$ values because they increase their waste flux to shed excess ingested protein. As a greater fraction of body nitrogen is excreted as ^{14}N-enriched waste, the body pool becomes ^{15}N-enriched (Macko et al. 1986; McCutchan et al. 2003; Sponheimer et al. 2003). In contrast, herbivores on a low-protein diet have lower $\Delta^{15}N$ values because they have a much lower waste flux and so retain more ^{14}N in tissues (McCutchan et al. 2003). In this case, the African lion and Canada lynx have the lowest $\Delta^{15}N$ values and are ingesting the diets with the lowest protein quantity as measured by their percent nitrogen values (weighted averages = 7.2% nitrogen and 10.5% nitrogen, respectively), whereas the bobcats and mountain lions have the highest $\Delta^{15}N$ values and are ingesting diets with slightly higher percent nitrogen values (weighted averages range from 11.6% nitrogen to 13.7% nitrogen). The differential in protein quantity ingested by the different felids could be contributing to their varying $\Delta^{15}N$ values. We saw similar patterns in fur from captive rats in that $\Delta^{15}N$ values were positively correlated with dietary protein quantity (C. M. Kurle, University of California San Diego, pers. comm.).

Another dietary related explanation for differences in the $\Delta^{15}N$ values observed among the felid species is that the $\delta^{15}N$ values of individual amino acids vary depending upon their source (Popp et al. 2007), similar to the $\delta^{13}C$ values of amino acids mentioned above. These source differences are reflected in the $\delta^{15}N$ values of the predator tissues because these tissues are composed of amino acids from the dietary sources. Therefore, differences in the $\delta^{15}N$ values of the amino acids that came from the protein sources in each diet could be contributing to the small variations observed in the $\Delta^{15}N$ values.

Conclusions.—Problems can arise in studies of stable isotope discrimination factors when animals are not exposed to an experimental diet for sufficient time to allow for previous dietary influences to clear from targeted tissues. Unlike soft tissues with continuous protein and isotope turnover, hair reflects diet consumed only during its growth. We are confident that the fur collected from animals in this study reflects the stable isotopes incorporated from their control diets. All samples were obtained after the the animal's yearly, early spring molt. In constructing the dietary stable isotope profiles for each animal, we followed 7 months of dietary records that spanned their time of molting.

Our study was conducted in a controlled setting; however, there were limitations that may have affected the accuracy of the results. The animals were kept in outdoor enclosures made of chain-link fencing. Wild prey would infrequently enter the enclosures and be eaten by the felids. Remains of lizards, frogs, squirrels, gophers, rabbits, and birds were found (rarely) in some of the enclosures. Such remains were not considered in the analysis of stable isotopes employed in our study. In addition, on rare occasions, an individual did not finish a meal or eat for several days. It is possible these minor fluctuations in the feeding schedule could have influenced the accuracy of our results.

Foraging ecology of wild animals is routinely estimated using stable isotope mixing models and the precision of these models relies on the input of accurate discrimination factors (Bond and Diamond 2011; Phillips 2012). Such factors, as described above, can vary considerably among species and diets. Therefore, studies using captive animals are crucial for deriving accurate estimations of animal diets. This may be especially important for large, relatively rare, elusive carnivores such as the felid species in the present study.

However, because our study included animals held on a variety of diets, some of which included a commercial carnivore diet that would not be representative of felid diets in the wild, we recommend the use of the mean $\Delta^{13}C$ (4.9 ± 0.5) and $\Delta^{15}N$ (4.1 ± 0.1) values obtained from the 3 bobcats using the stable isotope values from the NLE prey. These bobcats were held on diets composed of 100% whole animals and animal parts (79% whole rats and mice and 21% beef ribs and turkey wings), which best reflects diets of wild felids and so will be most applicable to reconstructions of wild felid diets. Finally, our use of a noninvasive tissue (fur) that can be collected opportunistically with no animal contact also is valuable for investigators studying the foraging ecology of felids in the wild.

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