



Original Article

What Are Snow Leopards Really Eating? Identifying Bias in Food-Habit Studies

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ABSTRACT Declining prey populations are widely recognized as a primary threat to snow leopard (*Panthera uncia*) populations throughout their range. Effective snow leopard conservation will depend upon reliable knowledge of food habits. Unfortunately, past food-habit studies may be biased by inclusion of nontarget species in fecal analysis, potentially misinforming managers about snow leopard prey requirements. Differentiation between snow leopard and sympatric carnivore scat is now cost-effective and reliable using genetics. We used fecal mitochondrial DNA sequencing to identify scat depositors and assessment bias in snow leopard food-habit studies. We compared presumed, via field identification, and genetically confirmed snow leopard scats collected during 2005 and 2012 from 4 sites in Central Asia, using standard forensic microscopy to identify prey species. Field identification success varied across study sites, ranging from 21% to 64% genetically confirmed snow leopard scats. Our results confirm the importance of large ungulate prey for snow leopards. Studies that fail to account for potentially commonplace misidentification of snow leopard scat may mistakenly include a large percentage of scats originating from other carnivores and report inaccurate dietary assessments. Relying on field identification of scats led to overestimation of percent occurrence, biomass, and number of small mammals consumed, but underestimated values of these measures for large ungulates in snow leopard diet. This clarification suggests that the conservation value of secondary prey, such as marmots (*Marmota* spp.) and other small mammals, may be overstated in the literature; stable snow leopard populations are perhaps more reliant upon large ungulate prey than previously understood. © 2016 The Wildlife Society.

KEY WORDS diet, DNA genotyping, feces, *Panthera uncia*, scat, snow leopard.

Lack of knowledge regarding the life history of a species is a common obstacle in creating conservation initiatives (Cisneros-Mata et al. 1995). For the snow leopard (*Panthera uncia*), knowledge of dietary habits is one such life-history component needed for effective conservation (Shehzad et al. 2012a). Knowledge of the relative composition of species in snow leopard diet will allow wildlife managers to design informed conservation initiatives. This is important, as it is estimated that there are 4,500–7,500 snow leopards in the wild and the species is listed in CITES Appendix I (Jackson et al. 2008, 2010). Most of the 12 countries in which they occur have banned their hunting (Jackson et al. 2008).

One major reason for the decline in snow leopard populations is a decrease in availability of natural prey due

to hunting, poaching, and competition with livestock (Shehzad et al. 2012a). In addition, when natural prey populations are low, snow leopards may turn to domestic livestock as a food resource (Mishra 1997). This can lead to retribution killing of snow leopards by herders, further exacerbating the strain caused by the low food supply (Mishra 1997). This further highlights the need to better understand snow leopard food habits as knowledge of snow leopard prey selection is essential for maintaining adequate numbers of prey on the landscape.

In general, studies that have relied solely on field identification of scat have found that snow leopards primarily eat ungulates (*Ovis* spp. and *Capra* spp.), but they will consume a considerable number of smaller prey such as marmot (*Marmota* spp.), hare (*Lepus* spp.), and pika (*Ochotona* spp.) (Oli et al. 1993, Lyngdoh et al. 2014). In a meta-analysis of snow leopard food habits, Lyngdoh et al. (2014) concluded that, in suboptimal conditions, snow leopards may switch to rely more heavily upon small mammals, particularly marmots, which may serve an important role in snow leopard conservation as a secondary

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food source, particularly in central China. However, Lyngdoh et al. (2014) based this on a single set of studies by Schaller et al. (1988) that did not account for potential identification bias of scat and thus, may have included scat samples from other sympatric carnivores. Similarly, Lovari et al. (2013) concluded that bharal (*Pseudois nayaur*) and marmots were important components of snow leopard diet, but their meta-analysis was also based on studies that did not perform genetic confirmation of scat origin. In contrast, genetically confirmed food-habits analyses tend to suggest a much greater reliance on large-bodied ungulate prey (Anwar et al. 2011, Shehzad et al. 2012a, Jumabay-Uulu et al. 2013, Lyngdoh et al. 2014).

Forensic scat analysis is a convenient, noninvasive approach to study snow leopard food habits (Oli 1994, Bagchi and Mishra 2006, Shrestha 2008). Although previous studies provide a baseline for snow leopard prey selection, many have assumed negligible error in the ability to discriminate snow leopard scats from those of sympatric carnivores—discrimination typically made using scat color, size, shape, and location, as well as secondary sign such as pugmarks, scrapes, or prey remains (Chundawat 1992, Oli et al. 1993, Bagchi and Mishra 2006, Shrestha 2008, Prasad Devkota et al. 2013). However, many sympatric carnivore scats have similar visual and physical characteristics (Hansen and Jacobsen 1999, Spiering et al. 2009, Shehzad et al. 2012a), and researchers may inadvertently include erroneous scat in their diet analysis, biasing their results. Mistaken field identification of scats, leading particularly to inclusion of red fox (*Vulpes vulpes*) scats in subsequent analyses, may in fact be commonplace, even among experienced biologists (Janečka et al. 2008). For example, Shehzad et al. (2012a) found snow leopard DNA in only 43.3% of putative snow leopard scats in Mongolia, similar to findings by Anwar et al. (2011; 51.6%) and Jumabay-Uulu et al. (2013; 67%). Genetic scat confirmation rates of <50% are not uncommon for other species (e.g., Koirala et al. 2012, Shehzad et al. 2012b), illustrating the broad challenges in distinguishing among sympatric carnivore scats. To remove identification bias, most recent studies of snow leopard food habits have relied on molecular methods to confirm scat species identity (Anwar et al. 2011, Shehzad et al. 2012a, Jumabay-Uulu et al. 2013, Kachel 2014), but many wildlife researchers continue to rely on field identification of scats, probably because of limited budgets and the increased costs associated with genetic methods (Lonsinger et al. 2015).

Many recent diet studies of both felid and canid species depended solely on field identification of scats (Brackzkowski et al. 2012, Kozłowski et al. 2012, Mondal et al. 2012, Lafferty et al. 2014, Etheredge et al. 2015, Kerley et al. 2015). Although previous studies have identified high error rates in field identification of scats (Anwar et al. 2011, Shehzad et al. 2012a, Jumabay-Uulu et al. 2013, Kachel 2014), the potential discrepancy between heuristic and molecular food-habits analyses has yet to be evaluated. This information may reinforce the importance of using genetic scat confirmation and also be useful when performing meta-analyses that rely on data obtained in studies that did not use

genetic scat confirmation (i.e., Lovari et al. 2013, Lyngdoh et al. 2014).

To gain insight into how bias may have impacted previous studies, we assessed the collection error bias in putative snow leopard scats collected from 4 sites in Central Asia. We used mitochondrial genetic sequencing to determine which scats came from snow leopards and subsequently compared conclusions about snow leopard food habits that were drawn from the naïve and genetically confirmed data sets.

STUDY AREA

Tajikistan

There were an estimated 180–300 snow leopards in Tajikistan, although these numbers were far from certain (McCarthy and Chapron 2003). We sampled scat from 2 study sites in the Pamir Mountains—Madiyan–Pshart and the Murghab Hunting Company Concession. Annual rainfall for the region averaged <100 mm of precipitation, falling mainly as snow. Average July temperatures ranged from 5° C to 10° C, and average January temperatures ranged from –15° C to –20° C. Potential wild snow leopard prey at the study sites included ibex (*Capra sibirica*), argali (*Ovis ammon*), marmots (*Marmota* spp.), and various small mammals (e.g., *Lepus* spp. and *Ochotona* spp.). Livestock grazing in the area consisted of domestic sheep (*Ovis aries*), goats (*Capra hircus*), cattle (*Bos taurus primigenius*), and yaks (*Bos grunniens*). Sympatric carnivores included gray wolves (*Canis lupus*), Eurasian lynx (*Lynx lynx*), red fox, and brown bears (*Ursus arctos*).

Madiyan–Pshart was an area roughly encompassed by 73°30'E to 73°45'E, 38°00'N to 38°20'N (Fig. 1). The landscape was characterized by the broad river valleys of the Murghab and Pshart drainages, surrounded by steep, nearly vertical, broken terrain, which was dissected by numerous subdrainages. There were herders' camps spaced approximately 5 km apart along the lengths of the central drainages; in summer, livestock operations could extend to well above 4,000 m in elevation. The town of Murghab (population 4,000) lay approximately 15 km east of the site.

The Murghab Hunting Company Concession in Jartygumbez lay approximately within the coordinates 74°15'E to 74°45'E, 37°30'N to 38°00'N. The Murghab Company used the area for guided commercial trophy hunting. Similar to the Madiyan area, the topography was defined by alternating broad valleys and steep mountains. Human settlements and seasonal camps, and associated livestock management, were far less common in the Jartygumbez area.

Kyrgyzstan

Snow leopard populations in Kyrgyzstan were estimated to be between 150 and 500 individuals (McCarthy and Chapron 2003). Kyrgyzstan had a similar climate to Tajikistan. Potential snow leopard prey included argali, Siberian ibex, Tolai hare (*Lepus tolai*), marmots, and various small mammals. Sympatric carnivores thought to inhabit the area included gray wolf, red fox, brown bear, stone marten (*Martes foina*), Eurasian lynx, Eurasian badger (*Meles meles*), and Palla's cat (*Felis manul*; McCarthy et al. 2010). Both

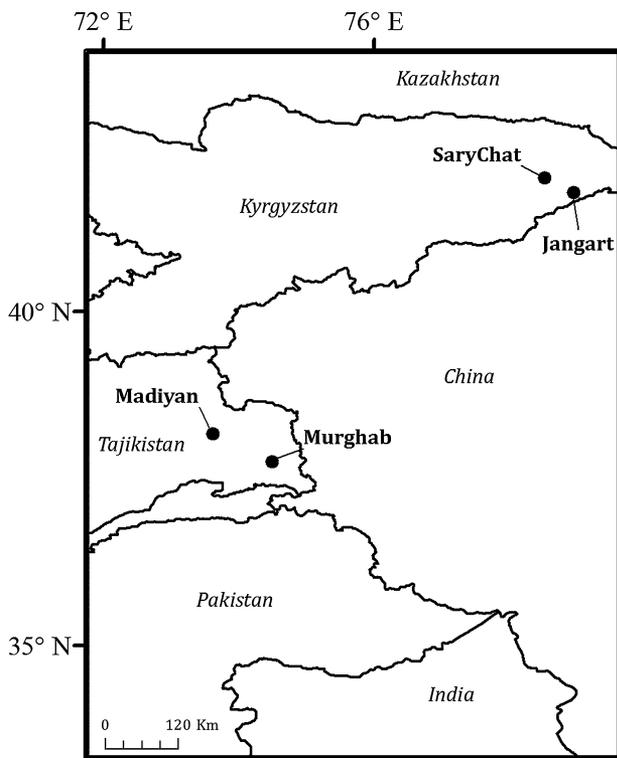


Figure 1. Map depicting the locations of 2 study sites in Kyrgyzstan from which snow leopard scats were collected between June and December 2005 (McCarthy et al. 2008), and 2 study sites in Tajikistan from which snow leopard scats were collected during summer of 2012 (Kachel 2014).

study areas in Kyrgyzstan were characterized by central river valleys rising steeply to peaks >4,000 m tall. Vegetation in the 2 areas was similar, consisting mostly of zerophytic grass and barren rock (McCarthy et al. 2008). The study areas were roughly encompassed by the coordinates 78°24'E to 78°34'E, 41°56'N to 42°4'N.

The SaryChat Ertash Zapovednik study site was a 720-km² protected area that was part of the Issyk Kul Biosphere Reserve (McCarthy et al. 2008), whereas the Jangart study area was located approximately 80 km southeast of SaryChat and close to the border with China. For many years, access to this politically sensitive border area was highly restricted; thus, it functioned similar to a protected area. At the time of sample collection, Jangart had recently become a hunting preserve. Although there were no permanent human settlements, hunting camps were set up by local guides and their clients (McCarthy et al. 2008).

METHODS

We analyzed 199 suspected snow leopard scat samples collected from Tajikistan and 56 scats collected from Kyrgyzstan. Tajikistan samples were collected during the summer of 2012 and Kyrgyzstan samples were collected between June and December of 2005. In Tajikistan, suspected snow leopard scats were collected opportunistically as they were encountered and small portions of each scat were placed in a 10-mL vial with 6 mL of silica desiccant (Kachel 2014). Scats were identified as snow leopard based on

location, size, and associated snow leopard signs such as pugmarks and scrapes. The vials were sent to the Center for Conservation Genetics at the American Museum of Natural History (New York, NY) for genetic sequencing in 2013. Extraction of DNA was performed using the QIAmp DNA Stool Mini Kit (Qiagen, Inc., Valencia, CA) following manufacturer's instructions, with adjustments to improve DNA quality and quantity. All samples were screened with species-specific primers amplifying parts of 4 mitochondrial genes (Cytochrome b, 12S rRNA, 16S rRNA, and ATPase-6). Genes were sequenced using an ABI 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, CA) and compared with the NCBI Nucleotide BLAST Database and an in-house database for mtDNA sequences (Benson et al. 2005). For more details, please see Caragiulo et al. (2013).

In Kyrgyzstan, suspected snow leopard scats were collected along systematic transects. Transects were chosen based on the most likely places to find snow leopard sign, such as along ridgelines (Jackson and Ahlborn 1989). Sixteen transects of variable length were walked once for 8.2 total km in SaryChat and 13 transects of variable length were walked once for 8.6 total km in Jangart. Scats were collected based on their size, shape, location, and surrounding signs and placed in 4 mL of 90% ethanol (McCarthy et al. 2008). The Laboratory for Conservation and Ecological Genetics at the Center for Research on Invasive Species and Small Populations, University of Idaho (Moscow, ID) performed genetic analysis on the samples in 2006. Extraction of DNA was performed in a laboratory specialized to handle low-quality DNA samples using the Qiagen stool kit (Qiagen, Inc.) following manufacturer protocols. Negative controls were used to monitor for contamination. This was followed by polymerase chain reaction (PCR) and sequencing of an approximately 160-base-pair region of the cytochrome B gene of the mitochondrial DNA control region using previously published primers and methods (Farrell et al. 2000, Onorato et al. 2006).

We used the portions of scats that were not sent for genetic sequencing to conduct our diet analysis. Most carnivores ingest at least a small quantity of hair with every meal, while bones and other indigestible structures are often avoided. Even when these structures are consumed, they generally become disrupted, and hair is the only prey remnant that remains intact enough for species identification (Brunner and Coman 1974). For this reason, we used hair features to identify prey.

The most important hair identification characteristic used was the medulla appearance. The medulla is the central core of the hair; different species produce medullas that look quite different. Cross-sections were not necessary to view the medulla patterns, because they were sufficiently visible using brightfield illumination to identify the prey species. As a secondary resource, we also examined the scale pattern left by the outer layer (the cortex) of the hair. Although scale patterns aid in hair identification, there are greater intraspecific variations and interspecific similarities than there are in medulla appearance (Brunner and Coman 1974). To enable comparison of medulla and scale-pattern

characteristics, we first removed hair samples from the scats with tweezers. We broke apart each scat and removed approximately 10–15 hairs from throughout each sample to maximize our potential to identify multiple species within one scat. Some scats had fewer than 10 hairs found. In these cases, we examined all of the hairs that were found. We manipulated the hairs slowly between the thumb and index finger of our nitrile gloves to remove any dirt and debris. If any debris remained attached to the hairs, we rinsed them with water and repeated the process, then allowed them to dry. To aid in microscopy of the hair samples, we painted microscope slides with clear nail polish and allowed them to dry for 10 min before placing the hairs onto the nail polish. This allowed the nail polish to set just enough that the hairs would not stick too tightly but still left an impression. We next placed a cover slide over the hairs and applied pressure to the slides overnight using c-clamps and wooden boards.

We examined the medulla and scale characteristics using standard microscopy techniques. We first examined the embedded hairs under the microscope to see the medulla pattern and then removed the hairs from the nail polish to examine the scale impression left behind. We compared both scale and medulla patterns against photographic keys (Brunner and Coman 1974, Shrestha 2008, Anwar et al. 2012) as well as scale patterns from known reference hairs. This allowed us to determine the prey composition of each scat.

We used a suite of prey composition metrics from the literature to assess how bias may have affected previous studies of snow leopard food habits (Chundawat 1992, Oli 1994, Bagchi and Mishra 2006, Shrestha 2008, Prasad Devkota et al. 2013). We first applied these metrics to all samples (i.e., those that were naïvely considered to be snow leopard upon collection). We then applied each metric to only the collected scats that were confirmed to be snow leopard after genetic sequencing. In both cases, we assessed food habits at each study site separately and then with data from all the sites combined, because differences in available prey might influence the results.

The most basic metric we calculated was the frequency of occurrence of prey items in the scat data. This was found by dividing the number of scats in which an item appeared by the total number of collected scats (Oli et al. 1993). The sum of all frequency of occurrence calculations may be >1 in some cases, because several scats contained hair from >1 prey species. We also calculated percent occurrence by dividing the total number of scats in which an item was found by the total number of items found (Anwar et al. 2011).

Frequency of occurrence or percent occurrence are not necessarily accurate measures of relative biomass of prey items consumed. This is because smaller mammals, such as rodents and lagomorphs, have a larger surface-area-to-volume ratio and thus, a greater percentage of the animal is not digestible (Jethva and Jhala 2004). When carnivores eat larger prey items, they can selectively consume the parts of the animal that are more digestible, resulting in fewer scats produced per amount of biomass consumed (Jethva and Jhala 2004). Additionally, consuming greater amounts of meat and

smaller amounts of hair or bones produces more liquid scats that would not be collectible in the field (Marker et al. 2003). Therefore, only looking at percent occurrence would overestimate the small mammal biomass consumed and underestimate the large mammal biomass consumed by a predator. To account for this problem, several previous studies performed feeding trials, in which they fed a carnivore specified amounts of prey items and counted the number of field-collectible scats produced (Floyd et al. 1978, Ackerman et al. 1984, Jethva and Jhala 2004, Marker et al. 2003). Using these data, they created an equation that related biomass of the prey species to the number of field-collectible scats produced per kilogram of prey species consumed, and discovered that the larger the prey biomass, the fewer field-collectible scats were produced per kilogram consumed (Ackerman et al. 1984). Although this type of study has not yet been performed for snow leopards, Bagchi and Mishra (2006) assumed that biomass of prey consumed to produce a single field-collectible scat was similar to that of cougars (*Puma concolor*), because their body sizes are similar.

To compare studies of snow leopard food habits that included a biomass-consumed formula, we followed the methods of Bachi and Mishra (2006). We used the average biomass of prey species to determine the kilogram of prey that would produce a single field-collectible scat, using the equation $Y = 1.98 + 0.035X$, where X = average body weight of prey species involved and Y = biomass of prey consumed to produce a single field-collectible scat (Ackerman et al. 1984). We obtained average body weights of prey species from the literature (Table 1; Oli 1994, Shrestha et al. 2005, Bagchi and Mishra 2006). We then estimated the amount of biomass of specific prey species consumed by snow leopards by multiplying the derived Y value by the number of scats found to contain that prey item. We further estimated the percentage of the diet by weight each prey species contributed by dividing the biomass of prey eaten by total biomass consumed.

Bagchi and Mishra (2006) concluded that large cats the size of snow leopards require 1.5 kg of food/day, hence 548 kg/year. However, because approximately one-third of ungulate biomass is inedible, they must actually kill 822 kg of biomass/year. We used this corrective factor to derive the

Table 1. Average body mass (derived from literature) of prey species used to estimate the kilograms of prey consumed to produce a single field-collectible scat during analysis of scat depositors and assessment bias in snow leopard food-habit studies.

Prey species	\bar{x} Body mass (kg)
Ibex (<i>Capra sibirica</i>)	76.0
Marmot (<i>Marmota caudata</i>)	5.3
Bird	1.5
Small mammal	0.5
Domestic sheep (<i>Ovis aries</i>)	35.0
Goat (<i>Capra hircus</i>)	34.0
Hare (<i>Lepus tolai</i>)	3.0
Argali (<i>Ovis ammon</i>)	93.7
Pika (<i>Ochotona roylei</i>)	0.2
Yak (<i>Bos grunniens</i>)	250.0

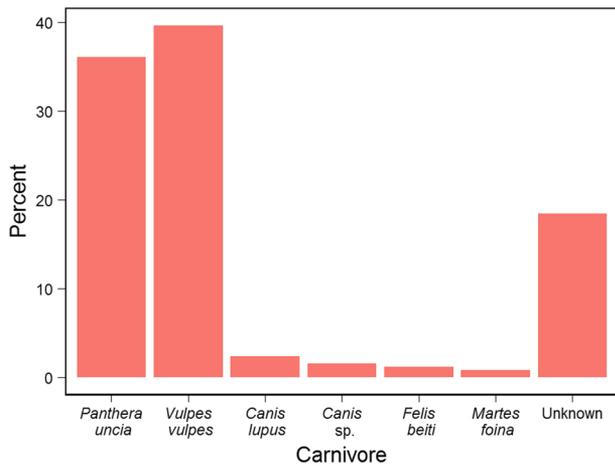


Figure 2. Percent of scats collected from 4 study areas in Tajikistan (2012) and Kyrgyzstan (2005) that initially were thought to be produced by snow leopards, but that were later confirmed via mitochondrial DNA sequencing to be produced by other carnivores.

number of individuals of each prey species required per year by snow leopards by multiplying 822 by the product of the percentage of biomass consumed and the biomass of the prey species.

Initially, we generated these estimates based on all of these data for individual prey species found in the scats. However, species composition varied among snow leopard habitat types; thus, to improve the inference of this study, we combined prey into 2 groups—small mammals and large ungulates. By categorizing prey into these groups, we were able to compare our results with studies performed in locations with different prey species. Using these groupings, we estimated 95% confidence intervals for the difference between confirmed snow leopard scats versus all collected scats for each calculation described above using bootstrapping with replacement with 5,000 replicates in Program R version 3.2.0 (R Core Team 2015).

RESULTS

Genetic confirmation of the samples from the 4 field sites ranged from 21% to 64%. Overall, 36.1% of collected scats were confirmed as snow leopard (92 scats—37 from the Murghab Hunting Company Concession, 22 from Madiyan-Pshart, 18 from Jangart, and 15 from SaryChat Ertash Zapovednik). Snow leopard fecal samples were most

often confused with red fox scat, which comprised 39.6% of collected scat samples (101 scats, Fig. 2). We identified 81.6% (208 scats) of the collected samples down to carnivore species using DNA analyses. The high amplification success rate was likely due to the arid and cold collection conditions, which may have frozen the samples and preserved the DNA (Murphy et al. 2007, Lonsinger et al. 2014). The majority of failed samples were from the Madiyan study site. This is potentially due to rainy weather conditions at the time of collection, which may have degraded the DNA. We included unidentified carnivore scats in the analyses of all scats but removed them when analyzing only snow leopard scats.

Most scats (78.3%) contained hair from only 1 prey species, although several had 2 or even 3 prey species (18.5% and 3.1%, respectively). When >1 species were detected, it was predominately hair from an ungulate species plus hair from a rodent or lagomorph. In total, we identified 10 prey species in the scats. We excluded 8 scat samples because prey hairs were too degraded to be identified ($n = 1$) or contained only snow leopard hair ($n = 7$), presumably from grooming. Many of the scats contained vegetation along with the hair. Most of the prey species were mammalian, although 7 scats contained bird feathers. For comparisons of all collected scats versus snow leopard scats, we focused only on the predominant mammalian prey, because all other prey items (birds, plants, etc.) were negligible.

Based on the change in value when removing scat other than confirmed snow leopards, use of all collected scats overestimated the frequency of occurrence, percent occurrence, percent biomass consumed, biomass consumed per year, and number of small mammals consumed, but analysis underestimated these measures for ibex and other large ungulates in snow leopard diet (Table 2). Confidence intervals for the change in value did not overlap 0 for any estimated variable when all data were combined (Table 2).

DISCUSSION

Lacking species validation of collected fecal samples (e.g., via genetic analysis), researchers likely include a large percentage of scats originating from non-target carnivores, thus biasing the results of their studies. In the case of Central Asian snow leopard populations, scat misidentification could potentially deemphasize the dietary importance of ungulates and underestimate the ungulate biomass required for a snow leopard population to persist. For example, using biased

Table 2. The median and 95% confidence interval change in analysis results when non-snow leopard scats (identified via mitochondrial DNA sequencing) were removed from each analysis for small mammals and large ungulates during an assessment of bias in snow leopard food-habits studies. Data from all sites (199 scats collected in Tajikistan during summer 2012; 56 scats collected in Kyrgyzstan between Jun and Dec 2005) were pooled to obtain these results.

Analysis	Prey type	Median	95% CI
Frequency of occurrence	Small mammals	-29.2%	-19.5 to -39.4
	Large ungulates	+24.2%	+15.9 to +32.3
Percent occurrence	Small mammals	-21.4%	-14.4 to -29.3
	Large mammals	+23.1%	+15.7 to +31.3
Percent biomass consumed annually	Small mammals	-11.7%	-6.5 to -17.0
	Large ungulates	+12.7%	+7.4 to +18.1
Relative no. consumed annually	Small mammals	-68.1 individuals	-45.5 to -89.5
	Large ungulates	+1.9 individuals	+1.0 to +2.9

results suggests that a hypothetical snow leopard population of 10 individuals in Tajikistan would require the biomass equivalent of 42 ibex and 24 argali/year. Using unbiased results, that same population would require the biomass equivalent of 57 ibex and 30 argali (Fig. 3). Given that under stable population conditions, these ungulates need to be surplus animals, the actual total ungulate population size required is even greater.

The high field-identification-error rates we observed (only 36.1% snow leopard feces) suggest that studies lacking genetic confirmation of scat may have skewed results for their diet analysis of snow leopards. We stress that we have no way of knowing if these previous studies had unmeasured collection bias (and are not implying that they did), but in the interest of discussion we have reviewed these studies to hypothesize what effects collection bias may have had on the results, if indeed it was present.

Several studies assumed accurate field identification of scats (based on size, shape, and nearby snow leopard signs such as scrapes and pugmarks) despite also reporting the local presence of various sympatric carnivores such as fox, gray wolf, wild dog (*Cuon alpinus*), stone marten, and Himalayan weasel (*Mustela sibirica*; Chundawat [1992], Oli et al. [1993], Bagchi and Mishra [2006], Shrestha [2008], Prasad Devkota et al. [2013]). Confusion with *V. vulpes* in many of these studies is possible, because the species is prevalent in the northern hemisphere and occurs throughout snow leopard range (Lariviere and Pasitschniak-Arts 1996, Macdonald and Reynolds 2008). Red fox have also been reported to occur at elevations as high as 4,500 m, which overlaps the elevations in which snow leopards are found (Aryal et al. 2010). These studies found that marmots and other small rodents accounted for 3.9–37.0% and ungulates comprised 65.2–80.5% of snow leopard diet using frequency of occurrence. Presuming error rates similar to our own and others (e.g., Janečka et al. 2008, Anwar et al. 2011, Shehzad et al. 2012a), it seems plausible that the other carnivore species reported in these studies could have been mistaken for

snow leopard, potentially overestimating the importance of rodents and small mammals. Moreover, Bagchi and Mishra (2006) reported 3.9% frequency of occurrence for marmots and rodents and mentioned that the only other carnivores active in the region were wolves, which do not utilize the same habitat as snow leopards and thus, are not likely to contribute to collection bias. This is consistent with the results of our study, in which gray wolf scats comprised only 2.4% of collected samples. This corroborates our finding that collection bias as a result of the species identified above can result in an underestimation of ungulates found in the snow leopard diet.

Studies that used molecular species identification to confirm carnivore origin reported increased occurrence of large ungulate detections and biomass in snow leopard diet compared with studies that relied solely on field identification. Jumabay-Uulu et al. (2013) found that ibex and argali comprised 80% of the diet; whereas Shehzad et al. (2012a) found that ungulates, including Siberian ibex, domestic goat, and argali, comprised 98.8% of the snow leopard diet; and Anwar et al. (2011) found that large mammals constituted 97.9% of the prey biomass consumed by snow leopards. Both Jumabay-Uulu et al. (2013) and Anwar et al. (2011) collected red fox scats most frequently after snow leopard scats.

Our findings call into question the validity of studies of snow leopard food habits based on scats that are not confirmed using genetic methods for identification. In particular, our results raise the possibility that erroneous field identification of scat may be the basis of the conclusion that marmots can be a locally or regionally important alternative food source when ungulate prey are scarce (Schaller et al. 1988, Lyngdoh et al. 2014).

To better understand how previous studies without genetic scat confirmation may be biased, it would be beneficial to examine prey densities in these study areas and determine the prey levels at which snow leopards may change their feeding habits (i.e., when do prey densities become low enough that snow leopards may switch to eating more small mammals). Moreover, identification of prey species by hair inspection may be an additional source of bias, because hairs from different species may look alike (Shehzad et al. 2012a). Therefore, future studies of snow leopard diet should also consider conducting genetic analysis on prey hairs removed from collected scats to obtain accurate results, although only analyzing hairs may not identify soft-bodied prey, well-digested prey, or small prey items such as bird species (Shehzad et al. 2012a). A possible solution to this might be using universal primers and next-generation sequencing, which Shehzad et al. (2012a) was able to use to successfully identify all prey in collected scats. Next-generation sequencing may also introduce its own bias, however, because it is not possible to tell what came from the stomach of the predator versus the gut contents of the prey (Jinbo et al. 2011). This may be a minor issue for snow leopards, because they are less likely to eat other carnivores. A more serious potential error may come from primer bias, where certain species may be preferentially amplified while others may be missed (Polz and Cavanaugh 1998, Pawluczyk et al. 2015).

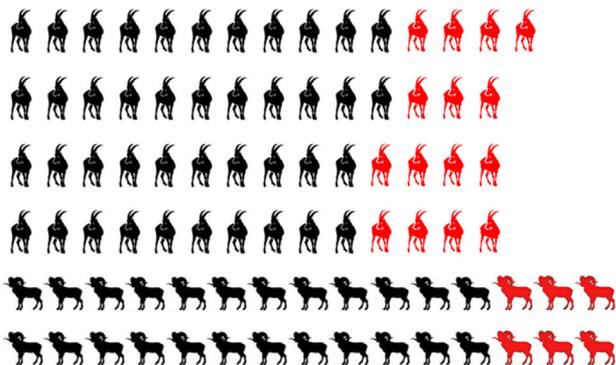


Figure 3. The biomass-equivalent number of prey animals (ibex and argali; black symbols) required annually by a hypothetical population of 10 snow leopards, as derived from biased food-habits data. When bias is reduced by removing scats of nontarget carnivore species identified via genetic sequencing, it becomes clear that 10 snow leopards would require additional prey animals (red symbols).

This problem may be mitigated by reducing the annealing temperature during PCR (Sipos et al. 2007).

MANAGEMENT IMPLICATIONS

To obtain accurate results, use of scat for diet studies of snow leopards and other species should utilize genetic scat confirmation and not rely solely on field identification. Our findings reinforce the strategy that in areas where food availability is limiting snow leopard populations, conservation efforts should aim to bolster wild ungulate populations and not rely on the questionable value of marmots and other small mammals as alternative prey. Increasing ungulate populations need not exclude trophy hunting; indeed, trophy hunting may be a viable option to increase the number of wild ungulates on the landscape. In Tajikistan and Pakistan, well-managed trophy hunting concessions had higher ungulate populations, likely because of a decrease in poaching (Woodford et al. 2004, Kachel 2014).

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