Diet of the brown bear in Himalaya: Combining classical and molecular genetic techniques

Muhammad Ali Nawaz[1,2,3,4]*, **Alice Valentini**[5,6], **Noor Kamal Khan**[3,4], **Christian Miquel**[5], **Pierre Taberlet**[5], **Jon E. Swenson**[2]

1 Department of Animal Sciences, Quaid-i-Azam University, Islamabad, Pakistan, **2** Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Norway, **3** Himalayan Wildlife Foundation, Islamabad, Pakistan, **4** Norwegian Institute for Nature Research, Trondheim, Norway, **5** Laboratoire d'Ecologie Alpine, Université Joseph Fourier, France, **6** Dipartimento di Ecologia e Sviluppo Economico Sostenibile, Università degli Studi della Tuscia, Viterbo, Italy

* nawazma@gmail.com

Abstract

The ecological requirements of brown bears are poorly known in the Himalaya region, which complicates conservation efforts. We documented the diet of the Himalayan brown bear (*Ursus arctos isabellinus*) by combining classical scat analysis and a newly developed molecular genetic technique (the *trn*L approach), in Deosai National Park, Pakistan. Brown bears consumed over 50 plant species, invertebrates, ungulates, and several rodents. Eight plant families; Poaceae, Polygonaceae, Cyperaceae, Apiaceae, Asteraceae, Caryophyllaceae, Lamiaceae, and Rubiaceae were commonly eaten with graminoids comprising the bulk of the diet. Golden marmots comprised the major mammalian biomass in the park, and were also the main meat source for bears. Animal matter, making 36% of dietary content, contributed half of the digestible energy, due to its higher nutritious value. We did not find a significant temporal pattern in diet, perhaps because the availability of the major diet (graminoids) did not change over the foraging period. Male brown bears were more carnivorous than females, probably because of their larger size, which requires higher energy and also makes them more efficient in capturing marmots. Frequencies of three plant species were also significantly higher in male brown bears; *Bistorta affinis*, *Carex diluta*, and *Carex* sp. Diet of the brown bear differed significantly between the park and surrounding valleys. In valleys, diet consisted predominantly of graminoids and crops, whereas the park provided more nutritious and diverse foodThe estimated digestible energy available to brown bears in Deosai was the lowest documented among brown bear populations, due to the lack of fruits and a relatively lower meat content. The low nutritious diet and high cost of metabolism in a high-altitude environment, probably explains the very low reproductive potential of this population.

Introduction

Knowledge of diet and foraging behaviour is important in the understanding of animal ecology and evolution, especially when they focus on broader nutritional interactions from an ecological perspective[1,2]. Such studies help identify key environmental resources required by a species, and thus enhance the understanding of habitat preferences and provide a knowledge base for successful management and conservation of wildlife populations. Due to growing recognition and methodological advancements, our understanding of nutritional ecology of bears has advanced significantly in the past two decades [3]. However, most diet studies of brown bear have been conducted in North America (e.g. [4–6]) or in Europe (e.g. [7,8]). The brown bear (*Ursus arctos*) is an opportunistic omnivore with a wide geographical distribution [9] and utilizes food according to local availability [10,11]. Therefore, the knowledge of brown bear diet from North America or Europe cannot be generalized for other geographical locations. There is limited information on the diet of brown bears in Asia [12–14] and in particular, no studies exist from the Himalaya, Karakoram and Hindu Kush ranges in South Asia. In order to plan and implement effective conservation programs for brown bears in Himalaya, a sound knowledge of nutritional ecology is essential [15].

The Himalayan brown bear (*U. a. isabellinus*) is distributed in small populations over the Himalaya, Karakoram, Hindu Kush, Pamir, western Kunlun Shan, and Tian Shan ranges in southern Asia [16]. They are highly threatened throughout their range due to poaching, habitat loss and fragmentation, yet their ecological requirements are generally not known. The reproductive rate is a critical factor in population viability of bears, because they have the slowest reproductive rate of any terrestrial mammal[17]. A long term monitoring study (1993–2006) of brown bears in Deosai National Park (DNP), Pakistan documented extremely low reproductive performance, due to late age of first reproduction (8.25 years), a long reproductive interval (5.7 years), and a small litter size (1.33) [18]. This study showed that the brown bear population in DNP is the least productive studied population in the world. A positive relationship between the diet of bears and their reproductive performance has been documented in a wide range of studies [19–23]. In North America, >90% of the variation in age of first reproduction was explained by vegetational productivity[24]. Autumn body mass, which is dependent on local food conditions, is an important indicator of reproductive output in bears [21–23].

The aim of our study was to document the diet of the brown bear in DNP in relation to its availability and contribution to energy assimilation. For this purpose; we assessed the availability of food resources, determined consumption of food by brown bears by combining classical scat analysis and molecular genetic techniques, and calculated the nutritional value of ingested food and its contribution to energy assimilation. We compared digestible energy (per unit of ingested food acquired) by brown bears in DNP with other brown bears in Asia and elsewhere.

We also investigated temporal and habitat effects, because seasonal and habitat variation in diet has been reported for brown bears [5,8,10,25,26]. Mattson suggested that gender-related nutritional needs may result in sex differences in diet [27]. Though not consistent in all studies [28–30], male bears often eat more meat than females [30–34]. We tested if sex-related differences exist in the selection of plant species or in overall diet items.

Materials and methods

Study area

The DNP occupies about 1800 km^2 of an alpine plateau in the western Himalayas and is managed administratively by the Northern Areas Forest and Wildlife Department, Northern

Areas, Pakistan. It is a typical high-altitude ecosystem, with mean daily temperatures ranging from –20˚C to 12˚C, and annual precipitation varying between 510 mm and 750 mm. It is above the timberline and vegetation is predominately herbaceous perennials, grasses and sedges. There are four kinds of habitats represented in the park; marshy, grassy, stony and rocky [35]. Marshy habitat is dominated by *Poa* and *Carex* spp., with some herbaceous plants. Grassy habitat is dominated by the Poaceace family, and stony habitat has great variety of herbaceous flowering plants. Rocky habitat is generally devoid of vegetation. Marshy habitats contribute most to the forage production, followed by grassy and stony vegetation habitats, whereas rocky areas are unproductive. The surrounding valleys have habitats distinct from the park (coniferous forest, shrubs, rocky and grassy slopes).

The park is covered by snow most of the year (generally October-May). Therefore, brown bears, which usually den in the surrounding valleys, come to DNP in June and leave in early October, when the snow returns. Most scat samples were collected in the park, but 43 were collected from the adjacent valleys, which provided insight into the diet of brown bears there.

Sample collection

We searched for bear feces throughout the study area from June to early October, during 2004–2005 and 2007. We divided the study area into five blocks, and searched each block for scats each year, covering most of DNP (see details in [36]). In addition, the DNP field staff collected scats during their normal patrolling of the park. For most of fecal samples, the date and location (Geographic latitude/longitude) were recorded using a Global Positioning System (GPS) receiver (Garmin 12XL). Scats were air dried and stored in polythene bags for analysis in the lab.

Samples for genetic analysis (1 cm^3) were collected in 20-ml plastic bottles with a stick of wood. Bottles were then filled with 95% alcohol to preserve the samples until DNA extraction. We also collected 112 plant specimens from Deosai and preserved them in silica gel. These plants were identified by taxonomists from the University of Karachi, Karachi Pakistan Museum of Natural History, Islamabad, and Quaid-i-Azam University, Islamabad.

Food availability

A total of 460 plant species have been identified from DNP, including 45 families and over 130 genera [37]. Asteraceae is the largest family, comprising 93 species, followed by Poaceae, 42 and Cyperaceae, 31. Other large families include Rosaceae, Schrophulariaceae, Polygonaceae and Fabaceae, with 25, 24, 23, and 22 species, respectively. For this study, we collected 112 plant species that were likely bear foods (based on field observations), 91 of those could be sequenced for whole chloroplast *trn*L (UAA [38], and 73 with identification at the species level were added to GenBank (accession numbers EU326032-EU326103, [39]. This reference database was used to identify plant sequences obtained from brown bear feces (see details below).

Slate-colored snow trout (*Diptychus maculatus*) and fleshy-mouthed snow trout (*Ptychobarbus conirostris*) are the only two fish species found in DNP [40] and were relatively abundant (pers. obs.). The ground-dwelling invertebrate fauna in DNP was sampled in 1999 [41]. It consisted of four classes, 13 orders and 102 determined families. Based on dry mass, five families dominated; Acrididae (24.6%), Tenebrionidae (13.7%), Lycosidae (11.7%), Carabidae (10.9%), and Anthrophoridae (9.4%).

Himalayan ibex (*Capra ibex sibrica*) and musk deer (*Moschus moschiferus*) occur in and around DNP, whereas the formerly common Ladakh urial (*Ovis orientalis vignei*) is locally extinct. We used field observations of the park staff, and surveys conduced in 2005 to estimate the populations of these ungulates.

Woods et al. recorded seven small mammal species in DNP (*Alticola argentatus*, *Sicista concolor*, *Sorex thibetanus*, *Hyperacrius fertilis*, *Marmota caudata* (golden marmot), *Mustela erminea*, *Ochotona roylei*) and provided their relative numbers [40]. *H. fertilis* is the most abundant species, followed by *M caudata* and *A. argentatus*. However all of these species are small (20–200 g weight), except for the golden marmot, which weighs ca. 3.5 kg [42] and comprises 97% of the biomass of rodents in DNP. From this and a study of activity patterns, which documented that bears dig out marmot colonies [43], we expected that marmots would be an important component of brown bear diet. Thus, we estimated the density of marmots in the park by walking 500-m wide line transects in 2004–2006. We walked along randomly placed transects, counted marmot colonies within the transects, and marked our routes with a GPS receiver. We plotted the routes of all transects on a map of the study area in ArcGIS (ESRI Inc. 2006) and calculated lengths. Colony densities were calculated from transect areas and multiplied by the average size of a social group (4.0 ± 0.22, [42]) to estimate marmot densities. In 2004, 14 transects were subdivided into habitat types, to calculate relative densities by habitat type. At each colony, we noted whether it had been dug out by brown bears to estimate accumulated brown bear impact on marmots.

Diet composition

Two life forms of plants, graminoids and herbs, dominate in Deosai and in the bears' diet. Therefore, it was difficult to differentiate diet components in scats on the basis of morphology. To overcome this limitation, we combined the classical scat analysis and a newly developed molecular technique (*trn*L approach, [44]) to identify diet components to a finer detail.

Scat analysis

We measured the volume of all scats before analysis by water displacement in a 2-l beaker. Scats were soaked and washed through a 0.8-mm mesh (same size used by Dahle [8]). We selected three sub-samples from this homogenized mixture and analyzed them in a petri dish under a 7–30 power stereoscope. We sorted diet components into nine categories; 1) rodents, 2) ungulates, 3) invertebrates, 4) graminoids, 5) forbs, 6) shrubs, 7) roots, 8) seeds, and 9) crops. Other infrequent items like fish and garbage were noted separately. Where possible we differentiated rodents into golden marmots and others. We estimated the percent relative volume (RV) of these diet categories visually, which is known to correspond well to actual volumes [45]. We calculated the Relative Frequency (RF) of each diet component as the total number of occurrences divided by the total scat samples.

Genetic analysis (the *trn*L approach): The 63 fecal samples were used in this study, which were previously typed by microsatellites [36]. Total DNA was extracted from about 10 mg of a feces sample with the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions. The DNA extracts were recovered in a total volume of 300 μL. Mock extractions without samples were systematically performed to monitor possible contaminations. DNA amplifications were carried out in a final volume of 25 μl, using 2.5 μl of DNA extract as a template. The amplification mixture contained 1 U of AmpliTaq® Gold DNA Polymerase (Applied Biosystems, Foster City, CA), 10 mM Tris-HCl, 50 mM KCl, 2 mM of MgCl$_2$, 0.2 mM of each dNTPs, 0.1 μM of each primer, and 0.005 mg of bovine serum albumin (BSA, Roche Diagnostic, Basel, Switzerland). The mixture was denatured at 95˚C for 10 min, followed by 35 cycles of 30 s at 95˚C,and 30 s at 55˚C; the elongation was removed in order to reduce the +A artefact [46,47]. Each sample was amplified with primers *g* and *h* [38], modified by the addition of a specific tag on the 5' end in order to allow the recognition of the sequences after the pyrosequencing, where all the PCR products from the different samples are mixed

together. These tags were composed of six nucleotides, always starting with CC on the 5' end, followed by four variable nucleotides that were specific to each sample.

PCR products were purified using the MinElute PCR purification kit (Qiagen GmbH, Hilden, Germany). DNA quantification was carried out using the NanoDrop® ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies® Wilmington, DE). Then, a mix was made taking into account these DNA concentrations in order to obtain roughly the same number of molecules per PCR product corresponding to the different feces samples.

Large-scale pyrosequencing was carried out on the 454 sequencing system (Roche, Basel, Switzerland) following manufacturer's instructions, and using the GS 20. From the mix of sequences obtained after the pyrosequencing, the first step in the data analysis consisted of dispatching the different sequences according to the tag present on the 5' end of the primers. Thus, for each sample (each feces), a file was generated, containing all the sequences having the relevant tag on its 5' end. Then, these sequences were analyzed to determine the diet. Only sequences present more than three times were taken into account in the subsequent analyses. To determine bear diet, the sequences were first compared to the reference database and then, if no match was found, to public databases, using the MEGABLAST algorithm [48].

We plotted the frequencies of identified families and classified them as regular ($\geq$10% occurrence) and occasional diet items (<10% occurrence) for brown bears. Families with >50% frequency were considered as preferred plant food for bears. Bellemain et al. found a significant negative correlation between the freshness of fecal samples and the proportion of positive amplification as well as between the freshness of fecal samples and the quality index [36]. In this study we tested whether the number of plant species identified from a sample were related to the freshness of the sample.

Energy contribution to the diet

Diet items differ greatly in their digestibility [6,49] and nutritional composition [50], which biases scat analysis. To adjust for differential digestibility of diet items, we estimated the Dietary Content (EDC) by applying Correction Factors (CF) proposed by [49] to RV. We used the following CFs: 4 for rodents, 3 for ungulates, 1.1 for invertebrates, 0.24 for graminoids and crops, 0.26 for forbs, 1 for roots, and 1.5 for seeds.

We estimated the energy contribution of each component of diet, by multiplying the EDCs by their respective estimated digestible energy values. For animal matter we used digestible energy values reported in [50]; ungulates = 29.4 kj/g, rodents = 22.1, and invertebrates = 17.7 [51]. The digestible energy (kj/g) for plants in DNP was estimated as; graminoids:11.8, forbs: 11.2, and shrubs: 12.2 [18].

Sex variation

Bellemain et al. [35] identified 28 individual bears from DNA in fecal samples. Because we used the same samples in the present study, we could investigate sex differences in diet. We ran a table analysis (PROC FREQ) in SAS (SAS Institute Inc.) and computed Fisher's exact text and odds ratios between sexes [52]. Fisher's exact test was chosen due to small sample size for individual diet categories.

Temporal variation

We grouped the data into four months (June through September) to investigate whether there was a temporal trend in diet selection. We had few samples for October, which we included in September. We tested only five categories (rodents, graminoids, forbs, roots, seeds) with > 10% overall frequency. Although food was a multicategory response, diet categories are not

mutually exclusive in one sample. Therefore we could not use a multicategory logit model [52]. We treated each category as a binary response and ran five logistic models for each diet category. Letting π denote probablity of finding a diet component, we tested temporal impact using equation 5.4.3 in Agresti [52]:

$$Logit\ (\pi) = \alpha + \beta_i^x;\ \text{x} = \text{factor of month with levels}\ i = 1, 2, 3, 4\ (\text{June}-\text{September})$$

We ran PROC GENMOD procedure in SAS to estimate the parameters. The probability of finding a particular diet component in each month ($\hat{\pi}_i$) was calculated as:

$$\hat{\pi}_i = \frac{e^{\alpha+\beta_i^x}}{1 + e^{\alpha+\beta_i^x}}$$

The samples used in the *trn*L approach were collected only between July to September. We counted the number of species and families in each group and compared them across the months.

Habitat variation

Deosai is represented by four vegetation classes (marshy, grassy, stony, rocky), and each class has its own characteristic floral species composition and cover [35]. We plotted the locations of fecal samples on a vegetation map in Arc GIS (ESRI Inc., 2006) to determine the habitat type they were found in (marshy, grassy, stony, rocky, and valley). Habitat differences in diet contents were investigated using logistic regressions, following the same procedure as described for the temporal variation.

Results

Mammalian biomass

A small population of Himalayan ibex was present in the hills east of DNP and in the surrounding valleys. We recorded 12 sightings and 20 signs (including one dead ibex) within the park in 1999–2005 and 4 sightings in the surrounding valleys in 2005. We estimated about 25–30 individuals within the park and 50–70 in the surrounding valleys of Bubind, Minimerg, and Karabosh. Musk deer prefer forests, so they were not present in the park. We counted 18 deer in 12 sightings on 7 transects in the surrounding valleys in 2005, where we estimated a population of 20–30. Thus, the biomass of wild ungulates in and around DNP was approximately 8 tons (1.4 kg per km^2 within the park area).

Based on 33 transects (271 km length) we conducted during 2004–2006, we estimated golden marmot density at 79.7±4.6 individuals per km^2. This density corresponds to a biomass of 250 kg/ km^2. The rocky habitat was generally devoid of marmot colonies, density was similar in grassy and stony habitats (20 and 18 colonies per km^2, respectively) but the highest in marshy habitat (26 colonies per km^2). The three habitats supporting marmots cover about 65% of the park [35]. Multiplying the biomass estimate by the total productive area resulted in an estimate of 250 tons of marmot biomass for the entire park (about 300 kg /km^2), which is about 60 times higher than the biomass of the largest mammal (brown bear).We recorded sign of brown bear digging at 33% of the colonies, a density of 6.7 dug colonies per km^2.

Diet composition and energy contribution

We analyzed a total of 334 brown bear scats collected over four years (101, 114, 49, and 70 in 2003–2005 and 2007, respectively). The average scat volume was 139 ml (SD: 52). Most of the scats (70%) were composed of only plant residues. Graminoids (grasses and sedges) had the

highest frequency (93%) and constituted the bulk (85%) of the scat residues (Table 1) and forbs had the second highest frequency, 52% (presence recorded by stems and inflorescence only). The volume of animal residues was only 4%, with rodents constituting most (88%) of it. About 30% of the rodent residues were those of golden marmots, and rest could not be identified. We found remains of fish in 2 scats, birds in 3, and 4 scats contained garbage (plastics and food packing).

We could not differentiate plant matter taxonomically by scat analysis beyond the general categories of graminoids and forbs. However, with the *trn*L approach, we found a total of 57 plant taxa in the bear feces, belonging to 50 genera and 29 families (Table 2). The *trn*L approach allowed us to identify 47% of the plants to species level, 74% to genera, 77% to tribe, 82% to subfamily, and all to family (Table 2). Thirty-one species sequences were identified from the reference database of plants from the DNP and the remaining 26 species were the closest matches from public databases.

The 57 plant species were not evenly represented in the diet; the frequencies ranged from 2–92%. About 70% of the identified species were represented by ≤ 3 samples, and among them 27 species were represented by single samples. There were only four species with occurrence in more than 50% samples; one unidentified species of Poaceae, two species of Cyperaceae (*Carex diluta*, *Carex* sp.), and one species of Apiaceae (*Heracleum candicans*). The unidentifed grass (subfamily Poideae) had the highest frequency (92%). The dietary diversity at the generic level was similar; *Carex*, *Heracleum*, and one Poaceae genus (unidentified) were the only genera represented in more than 50% of the samples. Among the 29 identified families, 14 were represented by only one sample. The regular plant diet (≥ 10% occurrence) of brown bears was comprised of only 8 families; Poaceae, Polygonaceae, Cyperaceae, Apiaceae, Asteraceae, Caryophyllaceae, Lamiaceae, and Rubiaceae (Fig 1). The first four families constituted the preferred diet, with more than 50% occurrence. We did not find any correlation between age of the sample (fresh, 2–3 days old, 1-week old) and number of plant species identified (Spearman $r = -0.5$, $P = 0.66$).

The relative contribution to the energy assimilation was almost equal for animal (54%) and plant (46%) components of the diet. Rodents (48%) and graminoids (33%) were the main sources of energy for bears. Ungulates (7.7%) and roots (7%) were second, and other components were not important. The energy gained by brown bears per gram of ingested food was estimated at 14.8 kj.

Table 1. Relative frequency (RF), relative volume (RV) and estimated dietary content (EDC) of diet items in brown bear scats from Deosai National Park, Pakistan.

		RF (%)	RV (%)	EDC (%)
Animal Matter		26.6	4.1	36.5
	Rodents	19.2	3.4	32.5
	Ungulates	6.9	0.5	3.9
	Invertebrates	6.9	0.1	0.2
Plant Matter		100.0	95.9	63.5
	Graminoids	92.8	85.3	48.5
	Forbs	51.5	0.9	0.6
	Shrubs	3.9	0.0	0.0
	Roots	20.1	4.3	10.2
	Seeds	24.6	0.4	1.3
	Crops	5.7	5.0	2.9

Table 2. A complete list of plant species identified by the _trn_L approach in the diet of brown bears in Deosai National Park, Pakistan.

Family	Species	Rank[1]	Frequency	Food Type	Identification source[2]	Comments
Actinidiaceae	Actinidia	Genus	0.02	Fruit	Public database	_Actinidia chinensis_ and _Actinidia deliciosa_ both species found in Pakistan
Adoxaceae	Adoxaceae	Family	0.02	Forb	Public database	Not recorded from Pakistan yet, but one taxon _Adoxa moschatellina_ is expected to occur.[a]
Apiaceae	Apioideae	Subfamily	0.05	Forb	Reference	Also known as Umbelliferae, represented in DNP by 18 species.[b]
Apiaceae	Heracleum candicans	Species	0.50	Forb	Reference	
Araliaceae	Araliaceae	Family	0.02	Forb	Public database	Not recorded from DNP, but three taxa (Aralia cachemirica, Hedera nepalensis, Schefflera bengalensis) are expected to occur in the area.[a]
Asteraceae	Leontopodium brachyactis	Species	0.02	Forb	Reference	This family is represented by 93 species in DNP, including this one.[b]
Asteraceae	Asteraceae	Family	0.23	Forb	Public database	
Brassicaceae	Thlaspi andersonii	Species	0.02	Forb	Reference	This species has been documented from DNP, along with other six species from this family.[b]
Caryophyllaceae	Cerastium cerastoides	Species	0.13	Forb	Reference	
Caryophyllaceae	Cerastium pusillum	Species	0.10	Forb	Reference	
Caryophyllaceae	Cerastium sp.	Genus	0.05	Forb	Reference	
Crassulaceae	Rhodiola sp.	Genus	0.02	Forb	Public database	
Cupressaceae	Cupressaceae	Family	0.03	Other	Public database	Three juniper species (_Juniperus communis, J. Excelsa, J. Turkestanica_) are documented from DNP.[b]
Cyperaceae	Carex diluta	Species	0.63	Graminoid	Reference	31 species of Cyperaceae, including Carex diluta, are documented from DNP.[b]
Cyperaceae	Carex	Genus	0.61	Graminoid	Public database	
Ephedraceae	Ephedra gerardiana	Species	0.02	Browse	Reference	Two species (_Ephedra gerardiana, E. Intermedia_) are present in DNP. Possible source for berries.[b]
Euphorbiaceae	Euphorbia sp.	Genus	0.02	Forb	Public database	Four species (_Ephorbia comigera, E. kanaorica, E. thomsonianum, E. Tibetica_) are documented in DNP.[b]
Euphorbiaceae	Euphorbiaceae	Family	0.02	Forb	Public database	
Fabaceae	Astragalus rhizanthus	Species	0.05	Forb	Reference	Also known as Papilionaceae.
Fabaceae	Oxytropis cachemiriana	Species	0.02	Forb	Reference	
Fabaceae	Galegeae	Tribe	0.03	Forb	Public database	
Fabaceae	Glycine sp.	Genus	0.02	Forb	Public database	
Griseliniaceae	Polysoma sp.	Family	0.03	Browse	Public database	
Juncaceae	Juncus sp.	Genus	0.02	Graminoid	Public database	Three species (Juncus articulatus, J. membranaceus, J. Sphacelatus) are recoded in DNP.[b]
Labiatae	Mentheae	Tribe	0.15	Forb	Reference	Either of Nepeta linearis or Thymus linearis are possible, because both have same molecular sequence.
Lycopodiaceae	Lycopodiaceae	Family	0.02	Other	Public database	Moss
Orobanchaceae	Pedicularis albida	Species	0.02	Forb	Reference	Scrophulariaceae
Orobanchaceae	Pedicularis sp.	Genus	0.02	Forb	Public database	
Papaveraceae	Papaver nudicaule	Species	0.02	Forb	Reference	
Pinaceae	Cedrus sp.	Genus	0.03	Tree	Public database	_Cedrus deodar_ is the only species in this genus, found in the surrounding valleys of DNP.[a]
Plantaginaceae	Plantaginaceae	Family	0.02	Forb	Public database	
Poaceae	Agrostis vinealis	Species	0.31	Graminoid	Reference	Poaceae is represented by 42 species in DNP.[b]
Poaceae	Elymus longi-aristatus	Species	0.23	Graminoid	Reference	Elymus longi-aristatus and Triticum (wheat) have same sequence, so wheat crop could be another possibility.

(Continued)

Table 2. (Continued)

Family	Species	Rank[1]	Frequency	Food Type	Identification source[2]	Comments
Poaceae	Koeleria macrantha	Species	0.05	Graminoid	Reference	
Poaceae	Poa alpina	Species	0.02	Graminoid	Reference	
Poaceae	Poa supina	Species	0.47	Graminoid	Reference	
Poaceae	Pooideae	Sunfamily	0.92	Graminoid	Public database	
Poaceae	Poa sp.	Genus	0.02	Graminoid	Public database	
Poaceae	Poa sp_91E	Genus	0.02	Graminoid	Public database	
Poaceae	Stipeae	Tribe	0.03	Graminoid	Public database	
Polygonaceae	Aconogonon rumicifolium	Species	0.23	Forb	Reference	23 species of Polygonaceae are present in DNP.[b]
Polygonaceae	Bistorta affinis	Species	0.47	Forb	Reference	
Polygonaceae	Polygonum cognatum	Species	0.03	Forb	Reference	
Polygonaceae	Rumex nepalensis	Species	0.18	Forb	Reference	
Polygonaceae	Polygonaceae	Species	0.34	Forb	Public database	
Ranunculaceae	Aconitum violaceum	Species	0.05	Forb	Reference	
Ranunculaceae	Thalictrum sp.	Genus	0.02	Forb	Public database	Two species (*Thalictrum alpinum*, *T. foetidum*) are documented in DNP.[b]
Rosaceae	Alchemilla sp_67E	Genus	0.02	Forb	Reference	
Rosaceae	Cotoneaster affinis	Species	0.02	Forb	Reference	
Rosaceae	Rosoideae	Sunfamily	0.05	Forb	Public database	
Rubiaceae	Galium boreale	Species	0.10	Forb	Reference	
Rubiaceae	Galium sp.	Genus	0.03	Forb	Reference	
Rubiaceae	Rubiaceae	Family	0.02	Forb	Public database	
Rutaceae	Rutaceae	Family	0.02	Other	Public database	Cultivated (citrus, etc)
Salicaceae	Salix sp.	Genus	0.02	Browse	Reference	
Saxifragaceae	Saxifraga flagellaris	Species	0.02	Forb	Reference	Represented by seven species in DNP.[b]
Saxifragaceae	Saxifraga hirculus	Species	0.06	Forb	Reference	

[1.] Level to which plant was identified

[2.] Source of identification for DNA sequences; Reference (database of 91 plants from DNP), Public databases for finding closest match (Zhang et al. 2000).

[a.] Flora of Pakistan (http://www.efloras.org/flora_page.aspx?flora_id=5)

[b.] [37].

https://doi.org/10.1371/journal.pone.0225698.t002

Sex differences in diet

Scat analysis of 43 samples, for which sex was known (Fig 2), indicated that the foraging behavior of the sexes was quite similar, except for rodents (P = 0.02, the Fishers's exact test). Females' likelihood of eating rodents was 84% lower than that of males (Odds ratio: 0.16).

Among the 62 fecal samples analyzed by the *trn*L approach, 21 belonged to females, 37 to males, and for 4 sex was not known. We identified 34 and 43 species from female and male samples, respectively. The ratio of graminoids to forbs did not differ significantly (χ^2: 0.24, P = 0.63) among sexes. Comparing individual species, the Fisher's exact test indicated significant differences in three plant species. The likelihood of eating *Bistorta affinis* (Odds ratio = 0.30, P = 0.02), *Carex diluta* (Odds ratio: 0.34, P = 0.03), and *Carex* sp. (Odds ratio: 0.24, P = 0.01) was significantly higher for males.
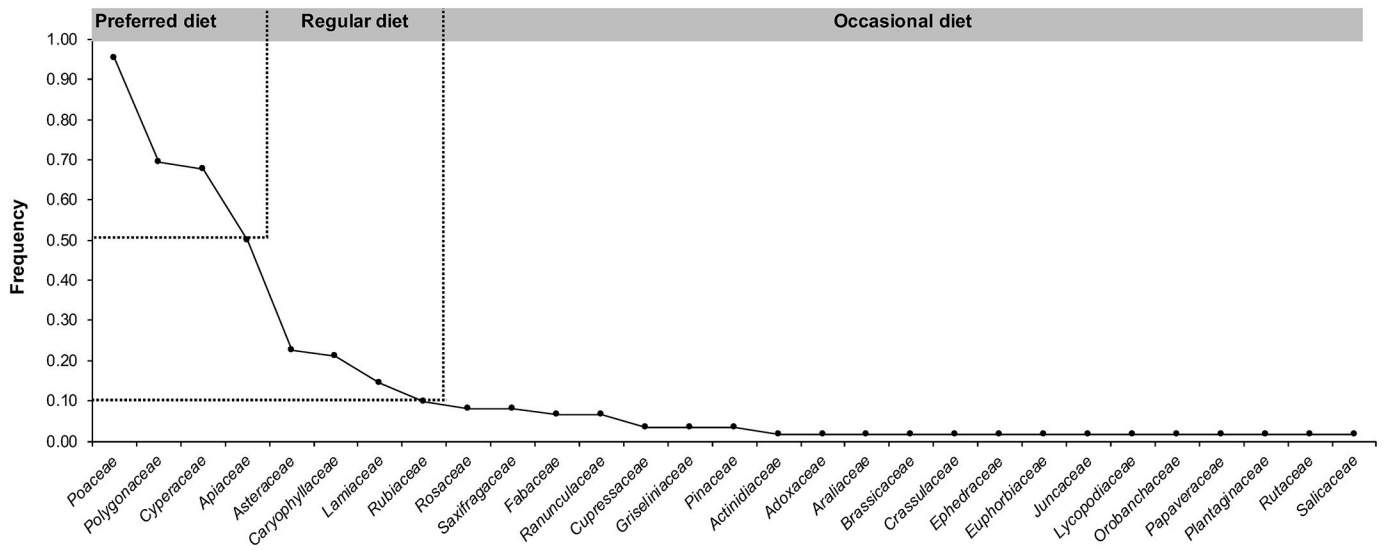
Fig 1. A frequency plot of plant families in the diet of brown bears in Deosai National Park, Pakistan, identified by the *trn*L approach.
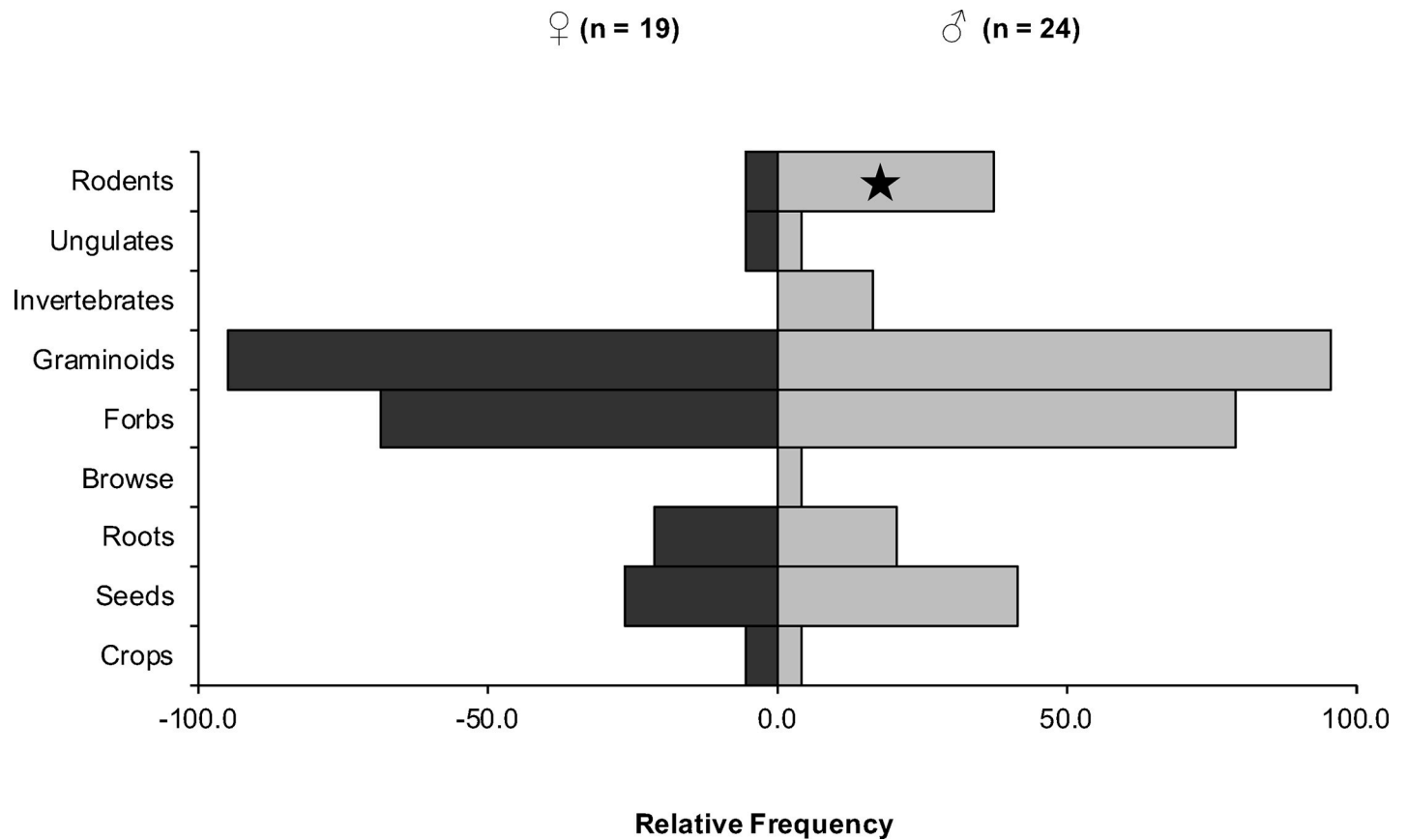
Relative Frequency

Fig 2. Sex differences in the diet of brown bears in Deosai National Park, Pakistan, based on scat analysis. Star indicates significant difference.
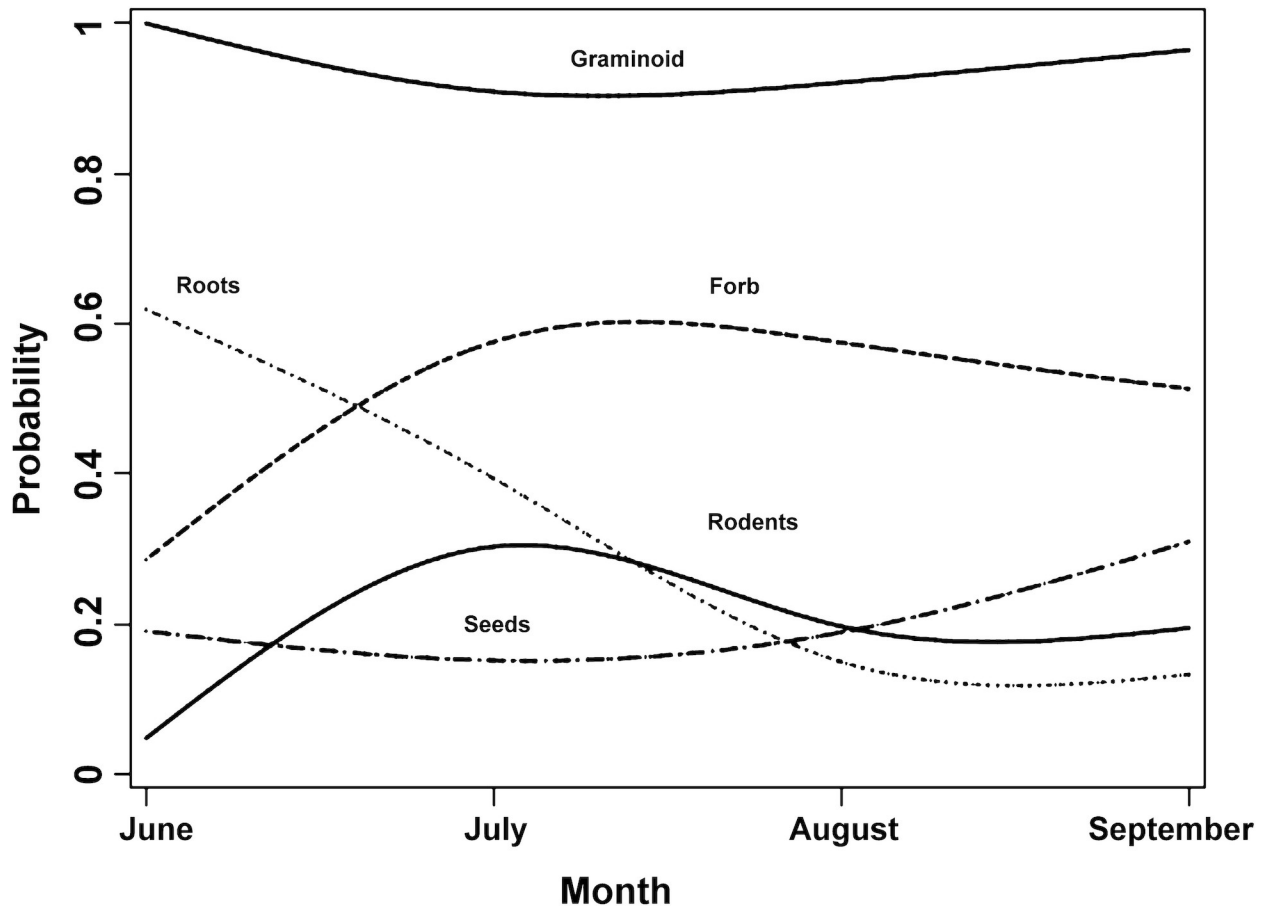
Fig 3. Temporal trend in probabilities of major diet categories of brown bears in Deosai National Park, Pakistan, based on scat analysis.

Temporal variation

The predicted probabilities of diet items depicted a divergent pattern (Fig 3). In the beginning of the season, the diet was dominated by graminoids and roots, and became more diverse in July. The frequency of roots was 10 times higher in June compared to September (exp (2.3624), Table 3). However, the logistic regressions indicated a lack of significant temporal

Table 3. Parameter estimates of logistic regression models of the temporal effect on major diet categories of brown bears in Deosai National Park, Pakistan. September was set as the base redundant parameter.

Parameter	Rodents	Graminoid	Forb	Roots	Seeds
Intercept	-1.4198*	3.3051*	0.0531	-1.8769*	-0.8014*
June	-1.5759	23.0603	-0.9694	2.3624*	-0.6456
July	0.5869	-1.0025	0.2523	1.4461*	-0.9214
August	0.0137	-0.8455	0.2484	0.1392	-0.6553
Model Fit	Good	Good	Poor	Good	Good
G²	285.92	124.71	399.89	267.85	311.51
P-value	0.56	1.00	0.00*	0.820	0.184

*P-value < 0.05

effect on major diet components, except for roots, which showed a decline in occurrence late in the season (Table 3).

Also, in the *trn*L data, we did not find a temporal difference in the number of plant species (χ^2: 2.54, P = 0.77) or families (χ^2: 2.2, P = 0.82). However, the ratio of graminoid forage to forbs changed significantly over three months (Spearman's r: -0.82, P = 0.04), favoring forbs later in the season. Four families showed a temporal trend; Asteraceae (r: -0.522) and Poaceae (r: -0.309) declined late in the season, whereas Polygonaceae (r: 0.714) and Fabaceae (r: 0.617) showed an increasing trend. The higher frequency of the latter two families might account for a higher frequency of seeds in the scats late in the season.

Habitat variation

The four habitats in DNP were homogenous with respect to diet contents of scats (Wald Statistics ranged 0.14–2.94 with P-values 0.15–0.70, for all parameters tested in logistic regressions). However, the diet in valleys (n = 43) was significantly different from DNP (n = 188). In the surrounding valleys, we found higher likelihood of eating graminoids (β = 2.0471, Wald Statistics = 30.83, P <0.01), and lower likelihoods for rodents (β = -3.127, Wald Statistics = 38.39, P <0.01), roots (β = -2.0305, Wald Statistics = 30.31, P <0.01), and seeds (β = -2.4563, Wald Statistics = 35.64, P <0.01). The frequency of forbs did not differ (Wald Statistics = 0.344, P = 0.55). Thus, DNP provided more nutritious and diverse food to bears than the surrounding valleys.

Of the 62 fecal samples used in the *trn*L approach, 15, 16, 13, and 7 were collected from marshy, grassy, stony, and rocky habitats within the park, respectively. Ten were from surrounding valleys and location of 1 sample was not recorded. Neither the number of species (χ^2:1.52, P = 0.82) nor the number of families (χ^2:1.85, P = 0.76) varied significantly across habitat types. However, four families, Adoxaceae, Araliaceae, Ephedraceae and Orobanchaceae, were represented by single samples and were present only in the valleys. Pinaceae and Cupressaceae also occur only in valleys, although the fecal samples were collected from the park. The ratio of graminoids to forbs in the diet did not vary significantly (χ^2:1.35, P = 0.72) among the four habitats of the park, however samples from the surrounding valleys showed a significantly higher proportion of graminoids (χ^2:24.4, P <0.01).

Comparing the classical scat analysis and the trnL approach

Forty-three scat samples, analyzed by both techniques, provided an opportunity to compare classical scat analysis and *trn*L approach. The frequencies of graminoids, forbs and shrubs obtained by the *trn*L approach were 98, 84 and 7%, respectively, compared with 93, 61 and 5%, respectively for the scat analysis. In the scat analysis, three samples lacked graminoids. Two of these samples were composed solely of crop residues and one was dominated by animal remains. Brown bears used three crops from the valleys surrounding DNP; wheat (*Triticum aestivum)*, corn (*Zea mays*), and barley (*Hordeum vulgare*), all of which belong to the Poaceae family. By adding these two crop samples to "graminoids" in the scat analysis data, the frequency of graminoids became identical in both methods.

There was a large difference in frequencies of forbs determined by the two methods. In the scat analysis, the frequency of forbs was dependent upon the identification of herbaceous plants based only on the occurrence of stems or inflorescences. Two other categories of diet; seeds and roots, likely also belonged to forbs. When we pooled these three categories, the frequency rose to 75%, but remained lower than the *trn*L frequency (84%). We conclude that the *trn*L approach verifies the findings of the scat analysis concerning graminoids and shrubs, but the scat analysis underestimated the occurrence of forbs due to relatively low volumes in the

scat (about 1%). Both methods agreed that the occurrence of forbs increased in the late season, and graminoids occurred at higher frequencies in the valleys.

Discussion

Diet composition

The *trn*L approach and classical scat analysis are complementary techniques, and together can provide a comprehensive understanding of feeding ecology of an omnivorous species such as brown bear. The *trn*L approach provided a more accurate descrption of plant diversity in the diet and its frequency. The scat analysis helped ascertain relative volumes of major diet groups, particularly the animal prey, which could not be determined by the *trn*L approach.

The brown bear diet was quite diverse in DNP, represented by 57 plant species, insects, ungulates and several rodent species. Poaceae, Polygonaceae, Cyperaceae, and Apiaceae are the commonly eaten families. However, the adjusted diet content indicated that only graminoids (represented by sedges and grasses) and golden marmots comprised the bulk of the diet. Golden marmots, though relatively low in frequency, had the highest contribution to digestible energy.

Food selection in animals is a function of availability, handling time, and quality [53,54]. However, in the case of omnivores, availability is the key factor in diet selection, because their food varies between relatively rare but high-quality animal matter and abundant low-quality vegetation [5]. Looking at plant and animal resources separately, we found consumption in accordance with availability. Graminoids comprised the highest biomass in park, followed by forbs [35]. Shrubs, which are restricted to narrow stream belts, are poorly represented in diet. Fruit plants are also not available in the park. There were three plants in the diet that could be a source of fruits for bears; *Ephedra gerardiana*, *Actinidia* sp., and an unidentifed species of Griseliniaceae, but these were represented by few samples (frequency <0.03). When Deosai National Park was established in 1993, there was no resident population of ungulates [55]. A small population of ibex was occasionally visiting, which has recently increased to 25–30 individuals and inhabits the eastern hills of the park. Therefore, there was no substantial and predictable ungulate prey available to the bears in the park. Domestic livestock were guarded by dogs and shepherds in DNP. The golden marmot represented the major biomass of available mammals and comprised the main component of animal matter in the diet. The DNP has a great variety of invertebrate fauna, the abundance of different groups changes seasonally, but a continuous supply is available [41]. They did not make a substantial part of the bear diet, probably because they did not occur in an aggregated form, like anthills in Sweden [56] or moth aggregation sites in North America [57], where they make a significant contribution to energy assimilation in brown bears.

The *trn*L approach indicated that scat analysis underestimated the occurrence of forbs in the diet of brown bears, at least by 10%. Likewise, we might have underestimated their volume in the scats, which is a limitation of scat analysis that has been reported earlier [58]. However, underestimation of the volume of forbs may not have been substantial, because the following observations support the conclusion of the scat analysis that graminoids comprised the bulk of the food. First, habitat use usually is determined by the distribution of the main food plants [59,60], though those plants might be eaten due to their greater availability rather than selective preference [12]. We documented that brown bears prefer marshy habitats in DNP [35]. The marshy habitat, with predominantly graminoid vegetation, has the highest biomass production in DNP (3919 kg dry matter/km^2). It covers only 15% of the park but produces half of its vegetation biomass [35]. Secondly, during a time budget study, bears were mostly observed in marshy habitats where their dominant activity was grazing [43]. Thirdly, the highest density

of brown bears occurs in the Black Hole area (central part of the park), which is predominantly a marshy habitat [18]. Thus, the graminoids are the most abundant and concentrated source of food for bears, and apparently the key factor explaining resource selection by brown bears in DNP. In agreement with our results, brown bears using alpine habitat in Alaska are heavily dependent on graminoids [61].

Vertebrates that depend on plant matter for their nutritional requirements exhibit digestive track modifications, either through compartmentalization of the for-e gut or an elaborate sac-culation of the hind-gut [62]. These specializations aid in the retention of digesta and harbor microbial populations that convert indigestible plant matter (cell wall components) into absorbable nutrients [62,63]. The brown bear possesses an anatomically simpler gastrointent-inal tract like other carnivores [62,64]. Although two adaptations, an extremely large intestine and bunodont molars, allow the brown bear to utilize plant matter more efficiently than other carnivores, it has a limited capacity for microbial digestion. To overcome the limitation of low digestibility, herbivores like perissodactyles and omnivores (raccoon *Procyon lotor*, pig *Sus scrofa*, etc) respond by increasing consumption [63,65]. This strategy sacrifices retention time but enables animals to utilize the cell contents. The most extreme adaptation to high intake (up to 6% of body weight) and low extraction (8±3 hours of retention) has been observed in the giant panda *Ailuropoda melanoleuca* [63,66]. The retention time of plant food in brown bears is also very short (7±0.8 hour, 48), however the relatively larger intestine may increase absorption [63,67]. The retention time in brown bear is 72% and 86% shorter than in horses and ruminants, respectively (retention times in horse and sheep/goat are 25 and 50 hours, respectively, [62,67,68] Although the digestion of structural carbohydrates is insignificant in brown bears due to fast passage [47], the loss of cell soluble is small (protein digestion is only 5% lower than in ruminants, [48]. The high intake rate of brown bears is supported by a time budget study in DNP [43], where bears were observed spending the largest part of the day for-aging (67% of daylight hours) and foraging was predominantly grazing (96.3%). Brown bears therefore would require a consistent source of large amount of vegetation, which is provided by the marshy habitats in DNP.

Brown bears are sexually dimorphic [9], males are about 50% heavier than females in DNP [18]. Larger body size increases the reproductive success of males through; 1) increasing chances of fertilization in a promiscuous mating system [10,69,70]because ejaculate volume is correlated with size [71], and 2) increasing social dominance, which increases access to repro-ductive females [10]. The more carnivorous food of males was probably an effect of their larger body size. Maintaining larger body size requires more energy, which is met by meat [19]. Golden marmots made up the major meat source in DNP and capturing requires much soil digging (soil heaps up to 1 m height can be observed in a marmot colony). Large and stronger bears might be more efficient in digging marmot colonies.

Seasonal variation in diet composition has been reported for brown bears in areas where the seasonal abundance of food changes considerably or bears shift their habitat seasonally [5,10,72]For example, in central Sweden; ungulates comprise the main diet in spring, whereas ants, forbs, and ungulates dominate in summer, and berries dominate the autumn diet [8]. In DNP, we did not find a significant temporal influence, probably because the availability of major food item (graminoids) did not change over the months. Graminoids in moist places (like marshy habitats in DNP) remain physiologically active, thus higher in protein content, even during post-growing season [4,73]. During the late growing season, before denning, bears show hyperphagia [74] and may increase their intake of high nutritious food (meat) if available [5]. Therefore, we expected higher consumption of meat (marmots) during the later months. Golden marmots are very sensitive to low body temperature and hibernate socially in a single hibernaculum [42], which prevents body temperatures falling below a critical

threshold through coordinated bouts of social thermoregulation [75,76]. Blumstein and Arnold [42] reported, from an area close to DNP, that above-ground activity of marmots becomes limited by the first week of September, and they start plugging burrows for hibernation by the second week of September. Although brown bears foraged until October in DNP, the limited activity by marmots probably explains the lack of increase in meat intake in later months.

Brown bears eat anthropogenic foods where they coexist with humans [9]. Human-related food in the present study was predominantly crops, and in a few scats, we found cultivated fruits (citrus, kiwi) and garbage (food packaging). Residues of ungulates in scat, may also belong to domestic livestock. Brown bears usually do not attack livestock in our study area, because livestock are guarded by shepherds and dogs. They might therefore have scavenged livestock carcasses. Brown bears steal yoghurt, which people keep in open bags of goat/sheep skins for drying, from villages and shepherd huts. The DNP has neither settlements nor agriculture within the park area. All communities and their cultivations are in surrounding six valleys [18]. The majority of brown bear dens are also present in those valleys. Thus, brown bears stay in the valleys in early spring, after denning, and they raid crops at that time.

In conclusion, the brown bear diet in DNP is predominantly based on carbohydrates, and the protein content was low compared with other brown bear populations with comparable data (Table 4). However, Westerterp and Kayser [77] suggested that carbohydrates are a better energy source than proteins at high altitudes, because of their low thermogenesis values (5–10% for carbohydrates, and 20–30% for proteins), and because they require less oxygen to metabolize, which is an advantage in the low-oxygen environment of high altitudes. A carbohydrate-rich diet increases the respiratory quotient, which thus provides high oxygen saturation in the blood [78].

Energy assimilation and life history

The positive role of meat in the reproductive performance of female brown bears is well documented [17,19,79]. Mast is the second most important source of protein and energy and is consumed by brown bears in most populations in large quantities. For example, berries make 82% of the autumn diet in central Sweden [8], and pine nuts make up to 45% scat volume in Yellowstone [80]. Brown bears with access to abundant salmon are also reported to feed extensively on fruits (87% fecal volume, [81]). Robbins et al. [15] documented that mixed diets (salmon and fruits) contribute to 72% higher growth in brown bears as compared to a meat-based diet, and this effect is most pronounced in small-sized bears. A comparison of six brown bear populations (Table 4), indicated that the reproductive rate was positively related to the amount of animal matter (r = 0.86), fruits (r = 0.74) and digestible energy (r = 0.66) in the diet, and negatively related to the amount of vegetation in the diet (r = -0.910).

The energy in the diet of 22 brown bear populations ranged between 16.9–26.6 kj/g (average = 22.5) (Table 4). The predominantly carnivorous populations, like two populations of the Tibetan Plateau [14,82], have higher levels of digested energy. The brown bear population in DNP, which lacks fruits in its diet and has relatively little meat, assimilates the lowest amount of energy per unit ingested food of all brown bear populations with comparable data (Table 4). High-altitude populations, with a low nutritious diet and facing extreme environmental conditions, are expected to have poor reproductive performance [17,24]. These factors probably contribute to the very low reproductive rates of the brown bear population in DNP [18].

The Central Asian populations, which are closer to the Himalayan brown bear genetically and geographically [16,83], have access to mast and consequently higher levels of food energy (Table 4). Thus, the poor nutrition of Himalayan brown bear in DNP cannot be generalized for its entire range. Brown bears in forested areas of Himalaya might have better nutrition

Table 4. Comparison of energy assimilation in the brown bear population of Deosai National Park with other brown bear populations from Asia, Europe and North America. Energy assimilated per gram of ingested food was calculated for these studies by applying correction factors [49] and energy estimates of food items [51] to relative percent volumes.

Study Area	Diet Composition (%Volume)			Energy*	Rep. Rate**	Reference
	Veg	Fruit	Animal	(kj/g)		
Asia						
Deosai National Park, Pakistan	95.9	-	4.1 [d,e,f]	14.8	0.23	Present study; [39]
Kekexili Nature Reserve, China	2	-	98 [d,e]	25.6		[14]
Chang Tang Reserve, China	26.2	-	73.8 [d,e]	22.8		[82]
Southern Hokkaido, Japan	72.3	17 [a]	10.7 [c,e,f]	20.9		[12]
Northern Hokkaido, Japan	48.3	46.2 [a]	5.5 [c,d,e]	19.3		[13]
Western Tian Shan, Central Asia	22	55.7 [a]	20.8 [d,e,f]	21.1		[85]
Northern Tian Shan, Central Asia	60.9	20.5 [a,b]	18.6 [e]	20.6		[85]
Caucasian Reserve, Russia	35	53 [a,b]	12 [e]	23.9		[85]
Eastern Sayans, Russia	28.9	38.7 [a,b]	32.4 [e,f]	23.5		[85]
Western Sayans, Russia	34.4	54.8 [a]	10.8 [d,e,f]	24.3		[85]
Far East, Russia	23.5	43.2 [a,b]	33.4 [e,f]	25.5		[85]
Europe						
Central Sweden	43.6	26.7 [a]	29.7 [e,f]	20.1	0.96	[8,86]
North-eastern Norway	20.9	38.1 [a]	41 [e,f]	25.1		[87]
Nord-Trøndelag, Norway	33.3	16 [a]	50.7 [e,f]	26.6		[8]
Central-south Norway***	25	39 [a]	36 [d]	20.5		[88]
Riaño National Hunting Reserve, Spain	45.5	40.6 [a,b]	13.9 [e,f]	24.4		[7]
Cantabrian Mountains, Spain	34.1	56 [a,b]	9.9 [e,f]	24.0		[89]
Yugoslavia (Croatia)	29.1	68.7 [a,b]	2.2 [e,f]	22.8		[58]
North America						
Northern Yukon, Canada	76.4	20.3 [a]	3.3 [d,e,f]	16.9	0.50	[24,25,90]
West-central, Alberta, Canada	65.5	21.9 [a]	12.7 [e,f]	21.3		[91]
Banff National Park, Canada	65	25 [a]	10 [e]	21.3	0.48	[4,31]
Flathead River Drainage, BC, Canada	52	29 [a]	19 [d,e,f]	22.6	0.85	[5,92]
Yellowstone 1973–74, USA	80.1	6.1 [a,b]	13.8 [c,d,e]	22.7	0.62	[6,93]

* average energy per gram of ingested food

**number of cubs/female/year

***Adjusted volume after applying correction factors

[a]soft mast

[b]hard mast

[c]fish

[d]rodents

[e]ungulates

[f]invertebrates

https://doi.org/10.1371/journal.pone.0225698.t004

than in DNP, because these areas have wild ungulates and a variety of fruit-bearing plants. For example, Schaller [84] reported frequencies of markhor (*Capra falconeri*) and ibex at 17% and 16%, respectively, in scats of brown bear from Chitral Gol and Baltoro (both locations in Pakistan). However, he concluded that graminoids comprised the bulk of brown bear diet there.

Acknowledgments

University, Islamabad) helped in identification of plants. Dr Muhammad Rafiq provided lab facilities for scat analysis at the Pakistan Museum of Natural History, Islamabad. Dr. Bjørn Dahle provided guidance for scat analysis. Ghulam Murtaza, Muhammad Yunus, and Doost Ali Nawaz assisted in scat analysis. Staff of the Gilgit-Baltistan Wildlife Department and the Himalayan Wildlife Foundation helped in sample collection. We are thankful to all.

Author Contributions

Conceptualization: Muhammad Ali Nawaz, Jon E. Swenson.

Data curation: Muhammad Ali Nawaz, Noor Kamal Khan.

Formal analysis: Muhammad Ali Nawaz, Alice Valentini, Noor Kamal Khan, Christian Miquel, Pierre Taberlet.

Investigation: Muhammad Ali Nawaz, Alice Valentini.

Methodology: Muhammad Ali Nawaz, Pierre Taberlet, Jon E. Swenson.

Supervision: Pierre Taberlet, Jon E. Swenson.

Writing – original draft: Muhammad Ali Nawaz.

Writing – review & editing: Pierre Taberlet, Jon E. Swenson.

References

1. Robbins CT. Wildlife feeding and nutrition. Academic Press; 1993.

2. Sih A. Effects of ecological interaction on forager diets: competition, predation risk, parasitism, and prey behavior., Pp. 182–211 in Diet selection an interdisplinary approach to foraging behavior. John Wiley & Sons; 2009.

3. Robbins CT, Schwartz CC, Felicetti LA. Nutritional ecology of ursids: a review of newer methods and management implications. Ursus. 2004; 15: 161–171. https://doi.org/10.2192/1537-6176(2004)015<0161:neouar>2.0.co;2

4. Hamer D, Herrero S. Grizzly Bear Food and Habitat in the Front Ranges of Banff National Park, Alberta. Bears Their Biol Manag. 1987; 7: 199. https://doi.org/10.2307/3872626

5. McLellan BN, Hovey FW. The diet of grizzly bears in the Flathead River drainage of southeastern British Columbia. Can J Zool. 1995; 73: 704–712. https://doi.org/10.1139/z95-082

6. Mealey SP. The natural food habits of grizzly bears in Yellowstone National Park, 1973–74. 1980. pp. 281–292.

7. Clevenger AP, Purroy FJ, Pelton MR. Food Habits of Brown Bears (Ursus arctos) in the Cantabrian Mountains, Spain. J Mammal. 1992; 73: 415–421. https://doi.org/10.2307/1382077

8. Dahle B, Sørensen OJ, Wedul EH, Swenson JE, Sandegren F. The diet of brown bears (Ursus arctos) in central Scandinavia: effect of access to free-ranging domestic sheep Ovis aries. Wildlife Biol. 1998; 4: 147–158. https://doi.org/10.2981/wlb.1998.017

9. Schwartz C, Miller S, Haroldson M. Grizzly bear, Pp. 556–586 in Wild mammals of North America: biology, management, and conservation. (Feldamer GA, Thompson BC and Chapman JA., eds.). Johns Hopkins University Press; 2003.

10. Craighead JJ, Sumner JS, Mitchell JA. The Grizzly bears of Yellowstone: their ecology in the yellowstone ecosystem 1959–1992. Washington, D.C.: Island Press; 1995.

11. Lefranc MNJ, Moss MB, Patnode KA, Sugg WCI. Grizzly bear compendium. Washington, D.C.; 1987.

12. Nomura F, Higashi S. Effects of food distribution on the habitat usage of a female brown bear Ursus arctos yesoensis in a beech-forest zone of northernmost Japan. Ecol Res. 2000; 15: 209–217. https://doi.org/10.1046/j.1440-1703.2000.00342.x

13. Ohdachi SAT. Food habits of brown bears in Hokkaido, Japan. 7th International Conference on Bear Research and Management. International Bear Association; 1987. pp. 215–220.

14. Xu A, Jiang Z., Li C, Guo J, Wu G., Cai P. Summer food habits of brown bears in Kekexili Nature Reserve. Ursus. 2006; 17: 132–137.

15. Robbins CT, Fortin JK, Rode KD, Farley SD, Shipley LA, Felicetti LA. Optimizing protein intake as a foraging strategy to maximize mass gain in an omnivore. Oikos. 2007; 116: 1675–1682. https://doi.org/10.1111/j.0030-1299.2007.16140.x

16. Nawaz MA. Status of the brown bear in Pakistan. Ursus. 2007; 18: 89–100.

17. Bunnell FL, Tait DEN. Population dynamics of bears __ implications. In: Fowler CW, Smith TD, editors. Dynamics of large mammal populations. New York: John Wiley and Sons; 1981. pp. 75–98.

18. Nawaz MA, Swenson JE, Zakaria V. Pragmatic management increases a flagship species, the Himalayan brown bears, in Pakistan's Deosai National Park. Biol Conserv. 2008; 141: 2230–2241. https://doi.org/10.1016/j.biocon.2008.06.012

19. Hilderbrand G V, Jacoby ME, Schwartz CC, Arthur SM, Robbins CT, Hanley TA, et al. The importance of meat, particularly salmon, to body size, population productivity, and conservation of North American brown bears. Can J Zool. 1999; 77: 132–138.

20. Jonkel CJ, Cowan IM. The Black Bear in the Spruce-Fir Forest. Wildlife Monographs. WileyWildlife Society; pp. 3–57. https://doi.org/10.2307/3830521

21. Rogers LL. Effects of food supply and kinship on social behavior, movements, and population growth of black bears in northeastern Minnesota. Wildl Monogr. 1987; 97: 1–72. https://doi.org/10.2307/3830545

22. Schwartz CC, Franzmann AW. Interrelationship of Black Bears to Moose and Forest Succession in the Northern Coniferous Forest. Wildlife Monographs. WileyWildlife Society; pp. 3–58. https://doi.org/10.2307/3830725

23. Stringham SF. Grizzly Bear Reproductive Rate Relative to Body Size. Bears Their Biol Manag. 1990; 8: 433. https://doi.org/10.2307/3872948

24. Ferguson SH, McLoughlin PD. Effect of Energy Availability, Seasonality, and Geographic Range on Brown Bear Life History. Ecography. WileyNordic Society Oikos; pp. 193–200. https://doi.org/10.2307/3683021

25. MacHutchon AG, Wellwood DW. Grizzly bear food habits in the northern Yukon, Canada. Ursus. 2003; 14: 225–235. https://doi.org/10.2307/3873022

26. Welch CA, Keay J, Kendall KC, Robbins CT. Constraints on frugivory by bears. Ecology. 1997; 78: 1105–1119. https://doi.org/10.1890/0012-9658(1997)078[1105:COFBB]2.0.CO;2

27. Mattson DJ. Causes and consequences of dietary differences among Yellowstone grizzly bears (Ursus arctos). University of Idaho. 2000.

28. Case RL, Buckland L. Reproductive characteristics of grizzly bears in the Kugluktuk area, northwest territories, Canada. Ursus. 1998; 10: 41–47. https://doi.org/10.2307/3873107

29. Powell RA, Zimmerman JW, Seaman DE (David E. Ecology and behaviour of North American black bears: home ranges, habitat, and social organization. Chapman & Hall; 1997.

30. Boertje RD, Gasaway WC, Grangaard D V., Kelleyhouse DG. Predation on moose and caribou by radio-collared grizzly bears in east central Alaska. Can J Zool. 1988; 66: 2492–2499. https://doi.org/10.1139/z88-369

31. Garshelis DL, Gibeau ML, Herrero S. Grizzly Bear Demographics in and around Banff National Park and Kananaskis Country, Alberta. The Journal of Wildlife Management. WileyWildlife Society; pp. 277–297. https://doi.org/10.2307/3803605

32. Hobson KA, McLellan BN, Woods JG. Using stable carbon (δ 13 C) and nitrogen (δ 15 N) isotopes to infer trophic relationships among black and grizzly bears in the upper Columbia River basin, British Columbia. Can J Zool. 2000; 78: 1332–1339. https://doi.org/10.1139/z00-069

33. Jacoby ME, Hilderbrand G V., Servheen C, Schwartz CC, Arthur SM, Hanley TA, et al. Trophic Relations of Brown and Black Bears in Several Western North American Ecosystems. J Wildl Manage. 1999; 63: 921. https://doi.org/10.2307/3802806

34. Mattson DJ. Use of ungulates by yellowstone grizzly bears Ursus arctos. Biol Conserv. 1997; 81: 161–177. https://doi.org/10.1016/S0006-3207(96)00142-5

35. Nawaz MA, Martin J, Swenson JE. Identifying key habitats to conserve the threatened brown bear in the Himalaya. Biol Conserv. 2014; 170: 198–206. https://doi.org/10.1016/j.biocon.2013.12.031

36. Bellemain E, Nawaz MA, Valentini A, Swenson JE, Taberlet P. Genetic tracking of the brown bear in northern Pakistan and implications for conservation. Biol Conserv. 2007; 134: 537–547. https://doi.org/10.1016/j.biocon.2006.09.004

37. Nawaz MA, Shah M, Zakaria V. Environmental baseline of Deosai National Park. Draft Report. Islamabad: Himalayan Wildlife Foundation; 2006.

38. Taberlet P, Coissac E, Pompanon F, Gielly L, Miquel C, Valentini A, et al. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. Nucleic Acids Res. 2007;35. https://doi.org/10.1093/nar/gkl987

39. Nawaz MA. Ecology, genetics and conservation of Himalayan brown bears. Ecology and Natural Resource Management. Norwegian University of Life Sciences. 2008.

40. Woods C. Biodiversity and conservation of the Deosai Plateau, Northern areas, Pakistan. In Biodiversity of Pakistan. Proceedings of International symposium, Pakistan Museum of Natural History and Florida Museum of Natural History (Gainesville), Islamabad, Pakistan. 1997.

41. Kok OB, Haddad CR, Niekerk VDJ, Butler HJB, Nawaz MA. Invertebrates as a Potential Food Source of Brown Bears on the Deosai Plateau, Northern Pakistan. Pakistan J Biol Sci. 2005; 8: 13–19. https://doi.org/10.3923/pjbs.2005.13.19

42. Blumstein DT, Arnold W. Ecology and Social Behavior of Golden Marmots (Marmota caudata aurea). J Mammal. 1998; 79: 873. https://doi.org/10.2307/1383095

43. Nawaz MA, Kok OB. Activity patterns of brown bears (Ursus arctos) on the Deosai Plateau, northern Pakistan. Suid-Afrikaanse Tydskr vir Natuurwetenskap en Tegnol. 2004; 23: 61–67. https://doi.org/10.4102/satnt.v23i3.194

44. Valentini A, Miquel C, Nawaz MA, Bellemain E, Coissac E, Pompanon F, et al. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. Mol Ecol Resour. 2009; 9: 51–60. https://doi.org/10.1111/j.1755-0998.2008.02352.x PMID: 21564566

45. Mattson DJ, Blanchard BM, Knight RR. Food habits of Yellowstone grizzly bears, 1977–1987. Can J Zool. 1991; 69: 1619–1629. https://doi.org/10.1139/z91-226

46. Brownstein MJ, Carpten JD, Smith JR. Modulation of non-templated nucleotide addition by Taq DNA polymerase: Primer modifications that facilitate genotyping. Biotechniques. 1996; 20: 1004–1010. https://doi.org/10.2144/96206st01 PMID: 8780871

47. Magnuson VL, Ally DS, Nylund SJ, Karanjawala ZE, Rayman JB, Knapp JI, et al. Substrate nucleotide-determined non-templated addition of adenine by Taq DNA polymerase: Implications for PCR-based genotyping and cloning. Biotechniques. 1996; 21: 700–709. https://doi.org/10.2144/96214rr03 PMID: 8891224

48. Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. Journal of Computational Biology. 2000. pp. 203–214. https://doi.org/10.1089/10665270050081478 PMID: 10890397

49. Hewitt DG, Robbins CT. Estimating Grizzly Bear Food Habits from Fecal Analysis. Wildlife Society Bulletin (1973–2006). WileyWildlife Society; pp. 547–550. https://doi.org/10.2307/3783342

50. Pritchard GT, Robbins CT. Digestive and metabolic efficiencies of grizzly and black bears. Can J Zool. 1990; 68: 1645–1651. https://doi.org/10.1139/z90-244

51. Johansen T. The diet of the brown bear (Ursus arctos) in central Sweden. MSc thesis at Norwegian University of Science and Technology, Trondheim,36pp. 1997.

52. Agresti A. Categorical data analysis. Wiley-Interscience; 1996.

53. Krebs JR, McCleery RH. Optimization in Behavioural Ecology. In: Behavioural ecology: an evolutionary approach. Blackwell Scientific Publications; 1991.

54. Manly BFJ, McDonald LL, Thomas DL, McDonald TL, Erickson WP. Resource Selection by Animals: Statistical Design and Analysis for Field Studies. Second. London: Kluwer Academic Publishers; 2002.

55. Deosai Brown Bear Project Final Report. Himalayan Wildlife Foundation Islamabad Pakistan. 1999.

56. Swenson JE, Jansson A, Riig R, Sandegren F. Bears and ants: myrmecophagy by brown bears in central Scandinavia. Can J Zool. 1999; 77: 551–561. https://doi.org/10.1139/z99-004

57. White D Jr., Kendall KC, Picton HD. Potential Energetic Effects of Mountain Climbers on Foraging Grizzly Bears. Wildlife Society Bulletin (1973–2006). WileyWildlife Society; pp. 146–151. https://doi.org/10.2307/3783951

58. Cicnjak L, Huber D, Roth HU, Ruff RL, Vinovrski Z. Food Habits of Brown Bears in Plitvice Lakes National Park, Yugoslavia. Bears Their Biol Manag. 1987; 7: 221. https://doi.org/10.2307/3872628

59. Clark JD, Clapp DL, Smith KG, Ederington B. Black bear habitat use in relation to food availability in the interior highlands of Arkansas. NCASI Tech Bull. 1999; 89–90. https://doi.org/10.2307/3872716

60. Costello CM, Sage RW. Predicting black bear habitat selection from food abundance under 3 forest management systems. NCASI Tech Bull. 1999; 106. https://doi.org/10.2307/3872724

61. Atwell G, Boone DL, Gustafson J, Berns VD. Brown Bear Summer Use of Alpine Habitat on the Kodiak National Wildlife Refuge. Bears Their Biol Manag. 1980; 4: 297. https://doi.org/10.2307/3872884

62. Stevens CE, Hume ID. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. Physiological Reviews. American Physiological Society; 1998. pp. 393–427. https://doi.org/10.1152/physrev.1998.78.2.393 PMID: 9562034

63. Van Soest PJ. Nutritional ecology of the ruminant. Ithaca, NY (USA) Cornell Univ. Press; 1994.

64. Davis DD. The giant panda: a morphological study of evolutionary mechanisms / Davis D. Dwight. Chicago, U.S.A.: Chicago Natural History Museum,; 1964. https://doi.org/10.5962/bhl.title.5133

65. Clemens ET, Stevens CE. A comparison of gastrointestinal transit time in ten species of mammal. J Agric Sci. 1980; 94: 735–737. https://doi.org/10.1017/S0021859600028732

66. Dierenfeld ES, Hintz HF, Robertson JB, Van Soest PJ, Oftedal OT. Utilization of bamboo by the giant panda. J Nutr. 1982; 112: 636–641. https://doi.org/10.1093/jn/112.4.636 PMID: 6279804

67. Koike S, Kasai S, Yamazaki K, Furubayashi K. Fruit phenology of Prunus jamasakura and the feeding habit of the Asiatic black bear as a seed disperser. Ecol Res. 2008; 23: 385–392. https://doi.org/10.1007/s11284-007-0399-3

68. Udén P, Rounsaville TR, Wiggans GR, Van Soest PJ. The measurement of liquid and solid digesta retention in ruminants, equines and rabbits given timothy (Phleum pratense) hay. Br J Nutr. 1982; 48: 329–339. https://doi.org/10.1079/bjn19820117 PMID: 6810917

69. Schenk A, Kovacs KM. Multiple mating between black bears revealed by DNA fingerprinting. Anim Behav. 1995; 50: 1483–1490. https://doi.org/10.1016/0003-3472(95)80005-0

70. Steyaert SMJG, Endrestøl A, Hackländer K, Swenson JE, Zedrosser A. The mating system of the brown bear Ursus arctos. Mammal Review. Blackwell Publishing Ltd; 2012. pp. 12–34. https://doi.org/10.1111/j.1365-2907.2011.00184.x

71. Erickson AW, Mossman H.W., Hensel R.J., Troyer W.A. The breeding biology of the male brown bear (Ursus arctos). Zoologica. 1968; 53: 85–105.

72. Hamilton AN, Bunnell FL. Foraging Strategies of Coastal Grizzly Bears in the Kimsquit River Valley, British Columbia. Bears Their Biol Manag. 1987; 7: 187. https://doi.org/10.2307/3872625

73. Graham DC. Grizzly bear distribution, use of habitats, food habits and habitat characterization in Pelican and Hayden Valleys, Yellowstone National Park (Doctoral dissertation, Montana State University-Bozeman, College of Letters & Science).

74. Nelson RA, Folk GE, Pfeiffer EW, Craighead JJ, Jonkel CJ, Steiger DL. Behavior, Biochemistry, and Hibernation in Black, Grizzly, and Polar Bears. Bears Their Biol Manag. 1983; 5: 284. https://doi.org/10.2307/3872551

75. Arnold W. Energetics of Social Hibernation. 1993. pp. 65–80. https://doi.org/10.1201/9780429040931-7

76. Arnold W, Heldmaier G, Ortmann S, Pohl H, Ruf T, Steinlechner S. Ambient temperatures in hibernacula and their energetic consequences for alpine marmots Marmota marmota. J Therm Biol. 1991; 16: 223–226. https://doi.org/10.1016/0306-4565(91)90029-2

77. Westerterp KR, Kayser B. Body mass regulation at altitude. European Journal of Gastroenterology and Hepatology. 2006. pp. 1–3. https://doi.org/10.1097/00042737-200601000-00001 PMID: 16357611

78. Hamad N, Travis SPL. Weight loss at high altitude: Pathophysiology and practical implications. Eur J Gastroenterol Hepatol. 2006; 18: 5–10. https://doi.org/10.1097/00042737-200601000-00002 PMID: 16357612

79. Reynolds H V., Garner GW, Reynolds H V. Patterns of Grizzly Bear Predation on Caribou in Northern Alaska. Bears Their Biol Manag. 1987; 7: 59. https://doi.org/10.2307/3872608

80. Kendall KC. Use of Pine Nuts by Grizzly and Black Bears in the Yellowstone Area. Bears Their Biol Manag. 1983; 5: 166. https://doi.org/10.2307/3872534

81. Fortin JK, Farley SD, Rode KD, Robbins CT. Dietary and spatial overlap between sympatric ursids relative to salmon use. Ursus. 2007; 18: 19–29. https://doi.org/10.2192/1537-6176(2007)18[19:dasobs]2.0.co;2

82. Schaller GB. Wildlife of the Tibetan steppe. University of Chicago Press; 1998.

83. Galbreath GJ, Groves CP, Waits LP. Genetic resolution of composition and phylogenetic placement of the Isabelline Bear. Available: www.legacy.com/louisville/

84. Schaller GB. Mountain Monarchs. Wild sheep and goats of the Himalaya. Chicago and London: The University of Chicago Press; 1977.

85. Vaisfeld MA, Chestin IE. Bears: distribution, ecology, use and protection. in Game animals of Russia and adjacent countries and their environment. Russ Acad Sci World Soc Prot Anim Moscow; 1993.

86. Sæther BE, Engen S, Swenson JE, Bakke Ø, Sandegren F, Saether B-E, et al. Assessing the Viability of Scandinavian Brown Bear, Ursus arctos, Populations: The Effects of Uncertain Parameter Estimates. Oikos. 1998; 83: 403. https://doi.org/10.2307/3546856

87. Persson IL, Wikan S, Swenson JE, Mysterud I. The diet of the brown bear Ursus arctos in the Pasvik Valley, northeastern Norway. Wildlife Biol. 2001; 7: 27–37. https://doi.org/10.2981/wlb.2001.006

88. Elgmork K, Kaasa J. Food Habits and Foraging of the Brown Bear Ursus arctos in Central South Norway. Ecography. WileyNordic Society Oikos; pp. 101–110. https://doi.org/10.2307/3682968

89. Naves J, Ferna A, Rodri C, Delibes M, Uría R. Brown bear food habits at the border of its range: a long-term study. J Mammal. 2006; 87: 899–908. Available: https://academic.oup.com/jmammal/article-abstract/87/5/899/899115

90. Mclellan B. Density-dependent population regulation in brown bears. in Density dependant population regulation of black, brown and Polar bears (Taylor M., ed.). International Association for Bear Research and Management, Missoula, Montana, USA; 1994.

91. Munro RHM, Nielsen SE, Price MH, Stenhouse GB, Boyce MS. Seasonal and diel patterns of grizzly bear diet and activity in west-central alberta. J Mammal. 2006; 87: 1112–1121. https://doi.org/10.1644/05-mamm-a-410r3.1

92. McLellan BN. Dynamics of a grizzly bear population during a period of industrial resource extraction. I. Density and age-sex composition. Can J Zool. 1989; 67: 1856–1860. https://doi.org/10.1139/z89-264

93. Schwartz CC, Haroldson MA, White GC, Harris RB, Cherry S, Keating KA, et al. Temporal, spatial, and environmental influences on the demographics of grizzly bears in the Greater Yellowstone Ecosystem. Wildl Monogr. 2006; 161.