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Ecology, Genetics and Conservation of Himalayan Brown Bears

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Himalayan Brown Bears

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ABSTRACT

Asian bears are greatly threatened due to the impact of human activities, yet there is a critical lack of knowledge about their status and requirements for survival, which complicates conservation efforts. This study documents ecological requirements, genetic structure and life history of Himalayan brown bears (*Ursus arctos*), which have fragmented and mostly declining populations in South and Central Asia.

Presently, brown bears in Pakistan are distributed over three main mountain ranges (Himalaya, Karakoram, and Hindu Kush), probably in seven populations. All of these populations are small and declining. Deosai National Park (DNP) supports the largest and most likely the only growing population (5% annual growth, based on the 14-years census). The genetic and field methods provided a population estimate of 40-50 individuals in DNP. The fecal DNA analysis revealed that the level of nuclear genetic diversity of the Deosai population was globally lower than brown bear populations that are considered to have a good conservation status, such as in Scandinavia or North America. However, in spite of the presence of a bottleneck genetic signature, the Deosai population has a moderate level of genetic diversity and is not at immediate risk of inbreeding depression. The DNP population has an exchange of individuals with neighboring populations in Pakistan and India, which is maintaining its genetic health.

The analysis of the diet of brown bears in DNP, combing classical and molecular genetic techniques, indicated a great diversity in food species. They 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. However, graminoids made up the bulk of the diet. Golden marmots (*Marmota caudata*) comprised the major mammalian biomass in the park, and were also the main meat source for bears. Animal matter, comprising 36% of the dietary content, contributed half of the digestible energy, due to its higher nutritious value. 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. The habitat analysis (by Ecological Niche Factor Analysis) revealed that bears avoided higher elevations and steeper slopes, and showed a higher preference for more productive parts of the park (marshy, grassy, and stony vegetation types). The marshy vegetation was the most preferred habitat, probably due to its highest forage production and highest density of golden marmots. Brown bears tolerated human structures, such as roads and camps, but strongly avoided grazing areas with higher livestock density.

We followed recognizable individuals from 1993 through 2006, and documented an extremely low reproductive rate in the Deosai population, due to late age of first reproduction (8.25 years), a long reproductive interval (5.7 years), and a small litter size (1.33). The family association (4.2 years) is the longest ever reported for brown bears and might have contributed to relatively higher survival of young. The reproductive rate of the Deosai population was the lowest yet documented for any brown bear population.

The estimated digestible energy available to brown bears in Deosai National Park was also the lowest yet documented for any brown bear population, due to the lack of fruits and relatively lower meat content in the diet. The poor quality of the diet and high cost of metabolism in a high altitude environment probably explain the very low reproductive potential of this population. The combination of poor intrinsic growth potential and exchange of individuals suggest that the observed population growth was a product of both reproduction and immigration.

The recovery of the brown bear population in Deosai is significant, because the species is declining throughout most of its range in South Asia. However, considering that the population is still small, has poor growth potential, and a relatively low genetic diversity, it requires a continuous field and genetic monitoring. Maintaining and improving the connectivity with adjacent populations in Pakistan and India will be of paramount importance for its long-term survival. Managing human resource use without adversely affecting the brown bear population has been a major management challenge in DNP, and seems to have been achieved. We recommend monitoring the numbers and distribution of livestock and conducting a detailed inventory of the rangeland to maintain sustainable stocking rates in future. Brown bear conservation efforts in South Asia must target reducing human-caused mortalities, particularly of adult females. Involvement of people can increase efficiency in conservation, in addition to reducing cost and conflicts. Environmental education is an important instrument to change perceptions and attitudes, and is vital to achieving synergy in conservation efforts.

SAMMENDRAG

Asiatiske bjørner er svært truet av menneskelig aktivitet, og bevaringen er vanskelig fordi det er for liten kunnskap om deres status og overlevelseskrav. Dette studiet dokumenterer de økologiske kravene, den genetiske strukturen og livshistorien til den himalayiske brunbjørnen (*Ursus arctos*), som i dag består av fragmenterte og stort sett minkende populasjoner i Sør- og Sentral-Asia.

Brunbjørnen i Pakistan har tilhold i tre hovedfjellkjeder (Himalya, Karakoram og Hindu Kush), og er antageligvis fordelt på syv populasjoner. Alle disse populasjonene er små og minkende. Den største og eneste økende populasjonen (5 % årlig vekst basert på tellinger over 14 år) er i Deosai Nasjonal Park (DNP). Basert på genetikk- og feltmetoder er populasjonen estimert til 40-50 individer i DNP. DNA-analyser fra bjørneekskrementer avdekket at den genetiske diversiteten var lavere hos Deosaipopulasjonen enn i bjørnepopulasjoner som er kjent for å ha en god bevaringsstatus, som i Skandinavia og Nord-Amerika. Til tross for tilstedeværelsen av en genetisk flaskehals, har Deosaipopulasjonen en moderat genetisk diversitet og er ikke utsatt for umiddelbar innavl. DNP-populasjonen utveksler individer med nabopopulasjoner i Pakistan og India, som opprettholder dens genetiske sunnhet.

Analyser av brunbjørnens diett i DNP ved bruk av klassiske og molekylære genetiske teknikker i kombinasjon, indikerer en stor diversitet i bjørnens føde. De konsumerte over 50 plantearter, invertebrater, hovdyr og flere gnagere. Åtte plantefamilier; Poaceae, Polygonaceae, Cyperaceae, Apiaceae, Asteraceae, Caryophyllaceae, Lamiaceae og Rubiaceae var vanlige i dietten. Graminoidene utgjorde likevel hoveddelen av dietten. Murmeldyr (*Marmota caudata*) utgjorde den største pattedyrbiomassen i nasjonalparken, og var også bjørnenes hovedkilde til kjøtt. Dyr inngikk i 36 % av diettinnholdet, og på grunn av deres høye næringsverdi, bidro de til halvparten av den fordøyelige energien. Hanner var mer kjøttetende enn binner, antageligvis på grunn av en større kropp, som krever mer energi, men som også gjør de mer effektive til å fange murmeldyr. Habitat analysene (økologisk nisjefaktoranalyse) viste at bjørner unngikk høyereliggende områder og bratte skråninger, og viste en høyere preferanse for mer produktive deler av parken (sump, gresseng og steinete vegetasjonstyper). Myrvegetasjonen var den mest foretrukne habitattypen, antageligvis på grunn av høy fórproduksjon og mange murmeldyr. Brunbjørner tolererte menneskelige strukturer som veier og leirplasser, men unngikk spesielt beiteområder med høy tetthet av husdyr.

Vi fulgte identifiserbare individer fra 1993 til og med 2006 og dokumentert en ekstremt liten reproduktiv rate i Deosaipopulasjonen, hvilket skyldtes sen alder for første reproduksjon (8.25 år), lange intervall mellom hver reproduksjon (5.7 år) og små kullstørrelser (1.33). Familietilknytningen (4.2 år) er den lengste som er beskrevet for brunbjørnen og kan ha bidratt til høyere overlevelse for bjørnungene. Deosaipopulasjonens reproduktive rate var den laveste som er beskrevet for alle brunbjørnpopulasjoner. Den estimerte fordøyelige energien som var til rådighet for

brunbjørnen i Deosai nasjonalpark var den laveste som er beskrevet for brunbjørnpopulasjoner, noe som skyldtes mangel på frukter og et relativt lite innslag av kjøtt i dietten. En diett med lavt næringsinnhold og den høye metabolske kostnaden i høyalpine miljøer, forklarer antagelig det lave reproduktive potensialet for populasjonen. Den lave iboende potensielle populasjonsveksten og utveksling av individer, indikerer at den beskrevne populasjonens vekst var et resultat av både reproduksjon og immigrasjon.

Fordi forekomsten av brunbjørn er avtagende i nesten hele Sør-Asia, er økningen i brunbjørnpopulasjonen i Deosai av stor betydning. Liten populasjonsstørrelse, lavt vekstpotensiale og lav genetisk diversitet krever imidlertid kontinuerlig oppfølging i felt og overvåking av den genetiske utviklingen i populasjonen. Opprettholdelse og forbedring av forbindelsen med tilstøtende populasjoner i Pakistan og India kommer til å ha stor betydning for overlevelsen på lang sikt. Forvaltning av menneskelig ressursbruk uten å påvirke brunbjørnpopulasjonen har vært en betydelig forvaltningsutfordring i Deosai nasjonalpark og ser ut til å ha vært vellykket. Vi anbefaler å overvåke antall husdyr og fordelingen av disse gjennom detaljerte inventeringer av beiteområdene slik at en i fremtiden kan opprettholde et bærekraftige beitetrykk. Bevaring av brunbjørn i Sør-Asia må fokusere på å redusere menneskeskapt mortalitet, særlig for binner. Involvering av befolkningen kan øke effekten av bevaringsarbeidet, i tillegg til å redusere utgifter og å dempe konflikter. Utdanning i natur- og miljøvern er en viktig faktor for å endre holdninger og oppfatninger, og vil være av grunnleggende betydning for å oppnå synergieffekter av bevaringsinnsatsen.

KHULASA

خلاصہ

ایشیاء میں ریچھوں کی بقاء انسانی آبادی اور طرز زندگی کی وجہ سے خطرات سے دوچار ہے۔ مزید براں انکی زندگی کی ضروریات کا علم انتہائی محدود ہے جو ان کی بقا کی کوششوں میں حائل ہے۔ ہم نے ہمالیائی بھورے ریچھ کی زندگی کی ضروریات، Genetics اور Life History کا مطالعہ کیا ہے۔ ہمالیائی بھورا ریچھ جنوبی اور وسطی ایشیاء کا باشندہ ہے لیکن اسکی تعداد بہت کم، بٹی ہوئی اور زوال پذیر ہے۔

پاکستان میں بھورے ریچھ کی تقریباً 7 آبادیاں ہمالیہ، قراقرم اور ہندوکش کے پہاڑی سلسلوں میں موجود ہیں۔ تاہم دیوسائی نیشنل پارک کے علاوہ تمام آبادیاں بہت چھوٹی اور سکڑ رہی ہیں۔ دیوسائی میں ان کی آبادی کا تخمینہ 40-50 جانوروں کا ہے۔ فضلہ کے جینیاتی (Genetic) تجزیہ سے پتہ چلا ہے، کہ ان کا جینیاتی تنوع باقی بھورے ریچھوں سے کافی کم ہے۔ تاہم bottleneck کے آثار کے باوجود ان کا جینیاتی تنوع درمیانے درجے کا ہے۔ بھورے ریچھوں کی آمدورفت دیوسائی نیشنل پارک اور قریبی پاکستانی اور بھارتی علاقوں کے درمیان موجود ہے۔ جس کی وجہ سے ان کی جینیاتی صحت برقرار ہے۔

دیوسائی میں بھورے ریچھوں کی خوراک بہت سی اقسام پر مشتمل ہے۔ وہ پچاس سے زیادہ قسم کے نباتات، غیر فقری جانور، چوپائے اور متعدد قسم کے چوہے کھاتے ہیں۔ پودوں کے آٹھ خاندان (Poaceae, Polygonaceae, Cyperaceae, Apiaceae, Asteraceae, Caryophyllaceae, Lamiaceae, Rubiaceae) عموماً ان کی خوراک کا حصہ ہوتے ہیں۔ تاہم خوراک کا بیشتر حصہ گھاس پھونس پر مشتمل ہے۔ سنہرے چوہے دیوسائی میں کثیر تعداد میں دستیاب ہیں اور ریچھوں کیلئے گوشت کا اہم ذریعہ ہیں۔ اگرچہ خوراک کا صرف 36% حصہ حیوانات پر مشتمل ہے لیکن ریچھوں کو نصف توانائی ان سے میسر ہے۔ نر ریچھ زیادہ گوشت خور ہیں جسکی وجہ ان کا نسبتاً بڑا جسم ہوسکتا ہے۔ کیونکہ بڑے جسم کیلئے زیادہ توانائی درکار ہوتی ہے اور وہ چوہوں کی کھدائی کیلئے زیادہ موثر ہوتے ہیں۔ دیوسائی میں ریچھوں کے مسکن کے تجزیے سے پتا چلا ہے کہ وہ زیادہ بلندیوں اور ترچھی ڈھلانوں سے اجتناب کرتے ہیں۔ نباتاتی طور پر زرخیز علاقے ان کے پسندیدہ مسکن ہیں۔ جوہڑ نما گھاس کے میدان (Marshy Habitat) سب سے زیادہ پسندیدہ ہیں۔ کیونکہ ان میدانوں کی نباتاتی پیداوار سب سے زیادہ ہے اور وہاں پر سنہری چوہوں کی تعداد بھی نسبتاً زیادہ ہے۔ ریچھ سڑکوں اور خیموں سے مانوس نظر آتے ہیں لیکن یہ مویشیوں کے چرنے والے علاقوں سے گریز کرتے ہیں۔

ہم نے چودہ سال (1993-2006) تک ریچھوں کا انفرادی طور پر مشاہدہ کیا ہے۔ جس سے پتہ چلا کہ ان کی تولیدی پیداوار انتہائی محدود ہے۔ وہ اوسطاً 8 سال کی عمر میں پہلا بچہ پیدا کرتے ہیں۔ بچوں کا درمیانی وقفہ 5.7 سال ہے اور پیدائش کے وقت اوسطاً 1.33 بچے پیدا ہوتے ہیں۔ ماں اپنے بچوں کو اوسطاً 4.2 سال تک ساتھ رکھتی ہے۔ اتنی لمبی رفاقت ریچھوں میں کہیں اور نہیں دیکھی گئی اور شاید یہ بچوں کی کم شرح اموات کا سبب بھی ہے۔ قدرتی طور پر بڑھوتری کی صلاحیت دیوسائی کے ریچھوں میں دنیا بھر کے بھورے ریچھوں کی نسبت انتہائی کم ہے۔ خوراک سے حاصل کردہ توانائی بھی دوسرے ریچھوں کی نسبت بہت کم ہے جس کی وجہ خوراک میں پھلوں کا فقدان اور گوشت کی کمی ہے۔ ہمارے خیال میں خوراک کی پست غذائیت اور انتہائی بلندی پر رہنے کی وجہ سے زیادہ توانائی کی ضرورت بھورے ریچھوں میں سست افزائش نسل کے اسباب ہیں۔ دیوسائی میں ان کی تعداد میں سالانہ 5% اضافہ ہو رہا ہے۔ جس کی وجہ افزائش نسل کے علاوہ دوسرے علاقوں سے ریچھوں کی آمد ہے۔

دیوسائی میں بھورے ریچھ کی آبادی میں اضافہ نہایت اہمیت کا حامل ہے کیونکہ حیوانات کی یہ نوع پورے جنوبی ایشیاء میں زوال پذیر ہے۔ انکی کم تعداد، قدرتی بڑھوتری کی مختصر صلاحیت اور نسبتاً کم جینیاتی تنوع جیسے عوامل کا تقاضہ ہے کہ اس نادر جانور کی نگرانی اور حفاظت جاری رکھی جائے۔ دیوسائی سے ملحقہ علاقوں میں ان کی آمدورفت کو برقرار رکھنا بلکہ بہتر بنانا ان کے مستقبل کی بقا کیلئے بہت ضروری ہے۔ دیوسائی میں مویشیوں کی تعداد کی نگرانی ہونی چاہئے اور خیال رکھا جائے کہ ان کی تعداد علاقے کی پیداواری صلاحیت سے تجاوز نہ کرے۔

جنوبی ایشیاء میں بھورے ریچھ کو معدومیت سے بچانے کے لیے ان کے شکار کو سختی سے روکا جائے۔ ان کی بقاء کی کاوشوں کو مقامی لوگوں کے اشتراک سے مزید موثر بنایا جاسکتا ہے۔

CONTENTS

LIST OF PAPERS

This thesis is based on following articles, which are referred to in the text by their Roman numerals:

Paper I: Nawaz, M. A. 2007. Status of the brown bear in Pakistan, Ursus 18:89-100.

Paper II: Bellemain, E., M. A. Nawaz, A. Valentini, J. E. Swenson, and P. Taberlet. 2007. Genetic tracking of the brown bear in northern Pakistan and implications for conservation, Biological Conservation 134:537-547.

Paper III: Nawaz, M. A., J. E. Swenson, and V. Zakaria. An increasing low-productive, high-altitude brown bear population in South Asia; a successful case of national park management. – Submitted.

Paper IV: Valentini, A., C. Miquel, M. A. Nawaz, E. Bellemain, E. Coissac, F. Pompanon, L. Gielly, C. Cruaud, G. Nascetti, P. Winker, J. E. Swenson, P. Taberlet. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the *trn*L approach. – Molecular Ecology Resources (in press).

Paper V: Nawaz, M. A., A. Valentini, N. K. Khan, C. Miquel, P. Taberlet, J. E. Swenson. Diet of the brown bear in Himalaya: combining classical and molecular genetic techniques. – Manuscript.

Paper VI: Nawaz, M. A., and J. E. Swenson. Habitat selection by brown bears in Deosai National Park, Pakistan and implications for park management. – Submitted.

1. INTRODUCTION

There is general agreement that biodiversity is under assault globally due to population growth and an ever-increasing use of natural resources (Lewton and May 1995; Whittaker et al. 2005). The species extinction rate has increased greatly in recent times, and mammals are the most vulnerable (Primack 2002). By adopting the Convention on Biological Diversity, many governments have acknowledged biodiversity conservation as a global concern and an integral part of the development process. However, achieving such a goal in developing parts of the world, like South Asia, is particularly challenging, due to large-scale poverty, an enormous population, and greater dependence on natural resources. In the Himalayan region of the South Asia, rangelands and livestock are dominant sources of subsistence and are the major cause of conflicts with the conservation of mammals (Mishra 2001). Consequently all large mammals are threatened with extinction in Himalaya. Carnivores are particularly vulnerable, because they 1) naturally exist in small populations, and population size is one of the best predictors of extinction (Pimm et al. 1988), 2) they have large spatial requirements and an adequate prey base, and 3) they are poorly accepted by the public, as they pose a threat to livestock. One such carnivore species is the brown bear (*Ursus arctos*), which has declined in numbers and distribution by more than 50% in Asia during the past century (Servheen 1990).

Among the eight species of bears, the brown bear has the most widespread distribution (Servheen 1990; Schwartz et al. 2003). They are found throughout most of the northern hemisphere, including the Palearctic and Nearctic. The brown bears' status throughout the world varies from threatened to common; hence they are categorized as LR (lc) in the 2004 IUCN Red List and placed in Appendix II of the Convention on International Trade in Endangered Species (CITES). The species is most endangered in Asia, where small isolated populations exist mostly in remote mountainous areas (Servheen et al. 1999). The Himalayan brown bear (*U. a. isabellinus*), a subspecies that represents an ancient lineage of the brown bear (Galbreath et al. 2007), is distributed over the Great Himalaya region. Brown bears are generally well studied in North America and Europe. However there is a critical lack of information about their status and requirements for survival in Asia (Servheen et al. 1999), which hinders conservation efforts.

The Deosai Plateau in northern Pakistan has long been recognized as the main stronghold of brown bears in the country (Schaller 1977; Rasool 1991; Roberts 1997; Nawaz 2007). Population surveys in 1993 revealed that there were not more than 20 individuals in Deosai (Paper III), which raised concerns for their survival and lead to the declaration of area as a national park. A conservation program was initiated by the Himalayan Wildlife Foundation in collaboration with the Northern Areas Forest and Wildlife Department to protect the population and its habitat in order to allow the population to recover. Small population size is a great concern in conservation biology, because such populations may go extinct, even while protected, due to their intrinsic limitations (Primack 2002). When population size drops below a threshold, populations become susceptible to genetic, demographic and environmental stochasticities (Shaffer 1981). The loss of genetic diversity, due to genetic drift and inbreeding, is a key concern for the viability of small populations. Evolutionary processes, such as mutations, migration, selection, and stochasticity are also fundamentally different from those in large populations. In small populations, the role of stochasticity increases and the impact of selection is limited (Frankham et al. 2002). Due to demographic stochasticity, variations in birth and death rates cause the population size to fluctuate randomly, and may reduce it further. Species with low reproductive rates, like brown bears (Bunnell and Tait 1981), are particularly susceptible to demographic stochasticity, because they require a longer time to recover (Primack 2002). Random variation in the biological and physical environment causes temporal clustering of birth and death rates, which would increase uncertainty in population size (Lacy 2000).

Unfortunately, these three stochastic factors act simultaneously and often drive the size of populations downward, which ultimately leads to extinction. Such populations would only recover if a careful program is implemented. Understanding the ecological requirements, life history, population size, and dynamic processes that affect the population is vital for formulating an effective recovery and management plan of small populations (Primack 2002). These considerations provided the motivation for this study.

1.1 Aims of the Study

1.1.1 Status and Distribution

Documenting the status and distribution of Asian bears has been identified as a priority action for conservation by the IUCN/SSC Bear Specialist Group (Servheen et al. 1999). We combined field surveys, interviews and non-invasive genetic techniques to answer following questions: *i*) What is current status and distribution of brown bears in Pakistan, and specifically the population size in Deosai National Park (DNP)?, *ii*) Are the Pakistani populations of brown bears isolated genetically and geographically?, *iii*) Is the population in DNP increasing?

1.1.2 Genetic Diversity

Bear numbers in DNP declined drastically to as low as 19 in 1993 (Paper III). Although the population in Deosai has been recovering gradually, due to strict protection and conservation efforts, the decline could have reduced the genetic variability considerably. As a consequence, this population might suffer from inbreeding, and its survival might be compromised. We used the increasingly popular non-invasive genetic technique (Taberlet et al. 1996; Taberlet et al. 1999), and aimed to answer the following questions: *i*) What is the level of genetic diversity in the Deosai population?, *ii*) Did the population suffer from a bottleneck at the genetic level and how long ago did it begin to decline?, *iii*) Are Deosai bears at risk of inbreeding depression?

1.1.3 Diet Selection and Methodological Developments

Knowledge of diet and foraging behavior is important in the understanding of animal ecology and evolution (Sih 1993). Diet 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. Resource selection is related to reproductive success in animals (McLoughlin et al. 2006), therefore an analysis of diet and habitat help understand the life history of a species.

Several methods, from field observations to microscopic and chemical analysis of feces have been developed to evaluate the composition of animal diets. All of these methods have limitations, and their results are generally not comparable (Shrestha and Wegge 2006). Some methods provide description of food items (e.g, Microhistological analysis, Sparks and Malechek 1968) but are tedious and still identify only part of the diet. Other methods (e.g; Stable-isotope analysis, Hilderbrand et al. 1996; Near Infrared Reflectance Spectroscopy, Foley et al. 1998) provide only an estimation of the nutritional components, and therefore may not be helpful in resource management, because actual food sources remain unidentified. Studying food habits in herbivores is particularly challenging, because of the limited reliability and practicality of the available methods (Barker 1986). In this study we aimed to develop a DNA-based universal method, which could give a reliable and precise description of herbivore's diet. We then combined this new technique with available classical methods to precisely document the diet of the brown bear in DNP in relation to its availability and contribution to energy assimilation.

1.1.4 Habitat Selection

One of the major reasons DNP was created was to protect a declining population of brown bears. Since the livelihoods of local communities were dependent on park resources, a zoning plan was introduced (Paper III). The zoning plan allowed the distribution of park resources among various competing interests, such as human uses and wildlife, in order to meet management goals. The ecological needs of brown bears were not known at that time, therefore the allocation of areas for bears was based on sightings of brown bears and subjective assessments. The brown bear population in the park is growing (Paper III), and at the same time the magnitude of public resource use is increasing. This demands better understanding of the available resources and habitat use by brown bears, for appropriate management in future. We assessed these resources and their spatial distribution, and investigated habitat selection by brown bears.

1.1.5 Life History

The fitness of an organism is influenced by life history traits, which often are flexible and vary with environmental conditions (Dingle 1990; Stearns 1992; Clutton-Brock 1988). Variation in energy and environmental conditions over a geographical range induce variation in life history (Stevans 1989; Rosenzweig and Abramsky 1993; Brown 1995). This geographic pattern of life history variation is not limited to interspecific relationships, as populations may also differ within a species' range (Ferguson and McLoughlin 2000). Habitat stability (i.e., the degree of its seasonality and predictability) and temporal stochasticity are the two environmental factors that have a major impact on life history (Southwood et al. 1974; Clark and Yoshimura 1993). Brown bears are found throughout most of the northern hemisphere and occupy a variety of habitats from tundra to temperate forests (Schwartz et al. 2003; Servheen et al. 1999), consequently their life-history traits are diverse (Dahle and Swenson 2003; Stringham 1990; Zedrosser 2006).

The life history of high-altitude brown bears has never been documented. However, in environmental extremes (high seasonality, low productivity and temporal stochasticity), a less productive life-history is expected (Ferguson and McLoughlin 2000; Boyce et al. 2002; den Boer 1968). The assessment of reproductive performance and survival of individuals is important, because these factors limit population growth (Schwartz et al. 2006). Therefore we estimated demographic parameters and factors affecting the viability of high-elevation brown bears. We are the first to have done this.

2. STUDY AREA

Although we surveyed other parts of Northern Pakistan to determine the status of brown bears in the country, DNP was the main focus during this study (Fig. 1). DNP (75° 27' N, 35° 00' E) is an alpine plateau of about 1800 km^2 east of Nanga Parbat Peak, Northern Areas, Pakistan. The central part of DNP is relatively flat (0-10° slope) at elevations between 3400-4000 m, whereas the peripheral areas are steeper (up to 50° slope), with elevations up to 5300 m. Mean daily temperatures range from –20°C to 12 °C. The annual precipitation is 510 mm to 750 mm, and falls mostly as snow (HWF, 1999). The vegetation is predominately herbaceous perennials, grasses and sedges. There are four kinds of habitats represented in the park; marshy, grassy, stony and rocky (Paper VI). 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 a 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).

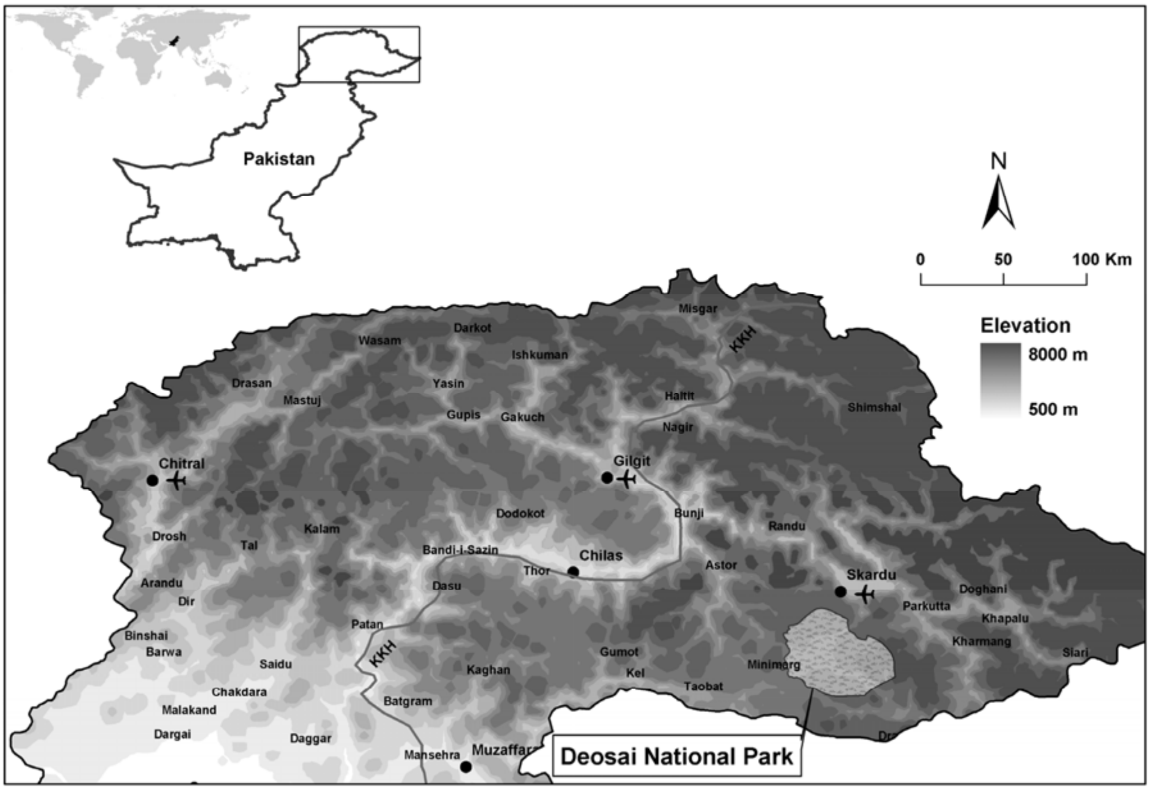

Figure 1: Location of Deosai National Park, Northern Areas, Pakistan.

The brown bear is the flagship species of the park; other mammals include Tibetan wolf (*Canis lupus chanco*), Himalayan ibex (*Capra ibex sibrica*), Tibetan red fox (*Vulpus vulpus montana*), golden marmot (*Marmota caudata*) and 17 other small mammal species (Nawaz et al. 2006). DNP is a typical highlands ecosystem, which is characterized by low atmospheric pressure, cold, aridity, low oxygen and carbon dioxide levels, intense isolation, rapid radiation, and high ultraviolet radiation (Mani 1990; Mani and Giddings 1980). The area has been dynamic climatically and geologically during the late Holocene (Kuhle 1997; Meiners 1997). The park is covered by snow most of the year (October-May, depending on weather). Therefore brown bears, which usually den in the surrounding valleys, come to DNP in June and leave in early October, when the snow returns.

DNP is a relatively flat area between narrow valleys and steep mountains, close to the Line of Control with India. Although there is no permanent habitation, because of the high altitude and extreme climate, there are many settlements along the periphery of DNP. They are located in numerous valleys and have various stakes in Deosai, especially traditional grazing rights. All but four peripheral communities utilize DNP's outer slopes and peripheral valleys for grazing. Four communities, Sadpara, Shilla, Dhappa and Karabosh, claim traditional grazing rights within the boundaries of DNP and their livestock occupy the eastern part of DNP during summer. In addition to these sedentary communities, there are nomad groups (*Bakarwals* or *Gujjars*), which come from the lowlands and compete for grazing resources. Approximately 9,000 livestock (belonging to resident and nomad communities), mainly goats and sheep, grazed within DNP in 2004.

3. MATERIALS AND METHODS

3.1 Methodological Advances

Working with a small population of a large carnivore in Himalaya required special considerations regarding methodology. We refined existing techniques, developed new methods, and used multiple approaches for each component of the study to ensure robust results (Fig. 2). For example we evaluated field-based population size estimates with a genetic method (Paper II), and combined classical scat analysis, molecular genetic technique, and stable-isotope analysis for understanding nutritional ecology. For genotyping of feces, we adopted a protocol developed for the Scandinavian brown bears (Bellemain and Taberlet 2004). The fecal samples from Himalaya were more degraded and less polymorphic, due to small population size (e.g., Mu10 and G10L were represented by only one or two alleles). Therefore we further developed that protocol by designating two new microsatellite primer pairs, namely G10J and G10H (Paper II). We ensured a high reliability of the genetic data by repeating amplifications (multi-tubes approach) and selecting samples with high quality-indices.

Two life forms of plants, graminoids and herbs, dominate in DNP, and in the bears' diet. Therefore differentiating diet components in scats on the basis of morphology was difficult. To overcome this limitation, we developed a new molecular technique (the *trn*L approach, Paper IV) to identify diet components to a finer detail. The *trn*L approach combines the plant barcoding concept (Chase *et al.* 2005, 2007) with the new highly parallel sequencing systems (Margulies *et al.* 2005). This method amplifes the P6 loop of the chloroplast *trnL* (UAA) intron (Taberlet et al. 2007) via the polymerase chain reaction (PCR; Mullis and Faloona 1987) and by subsequently sequencing individual molecules of this PCR product on the 454 automated sequencer (Roche Diagnostic, Basel, Switzerland). This method is very robust, fast, simple to implement, and broadly applicable to potentially all herbivorous species eating angiosperms and gymnosperms, from mammals to insects, birds and mollusks. The *trn*L approach represents a significant breakthrough in plant identification when using fecal material. We also introduced an approach to link DNA-based individual identification, the trnL approach, scat analysis and stable-isotope analysis for investigating the diet of an omnivorous species

3.2 Sample Collection

Three components of the study; genotyping, diet, and habitat use, were based on scat samples (Fig. 2), which were collected between 2003-2007. We divided the study area into five blocks, and searched each block for bear scats in order to cover most of the park. Transects were placed in each block, and walked by a team of 2-3 people. Apart from this planned collection, the field staff of DNP also collected samples during their normal patrolling in the park. For most of 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.

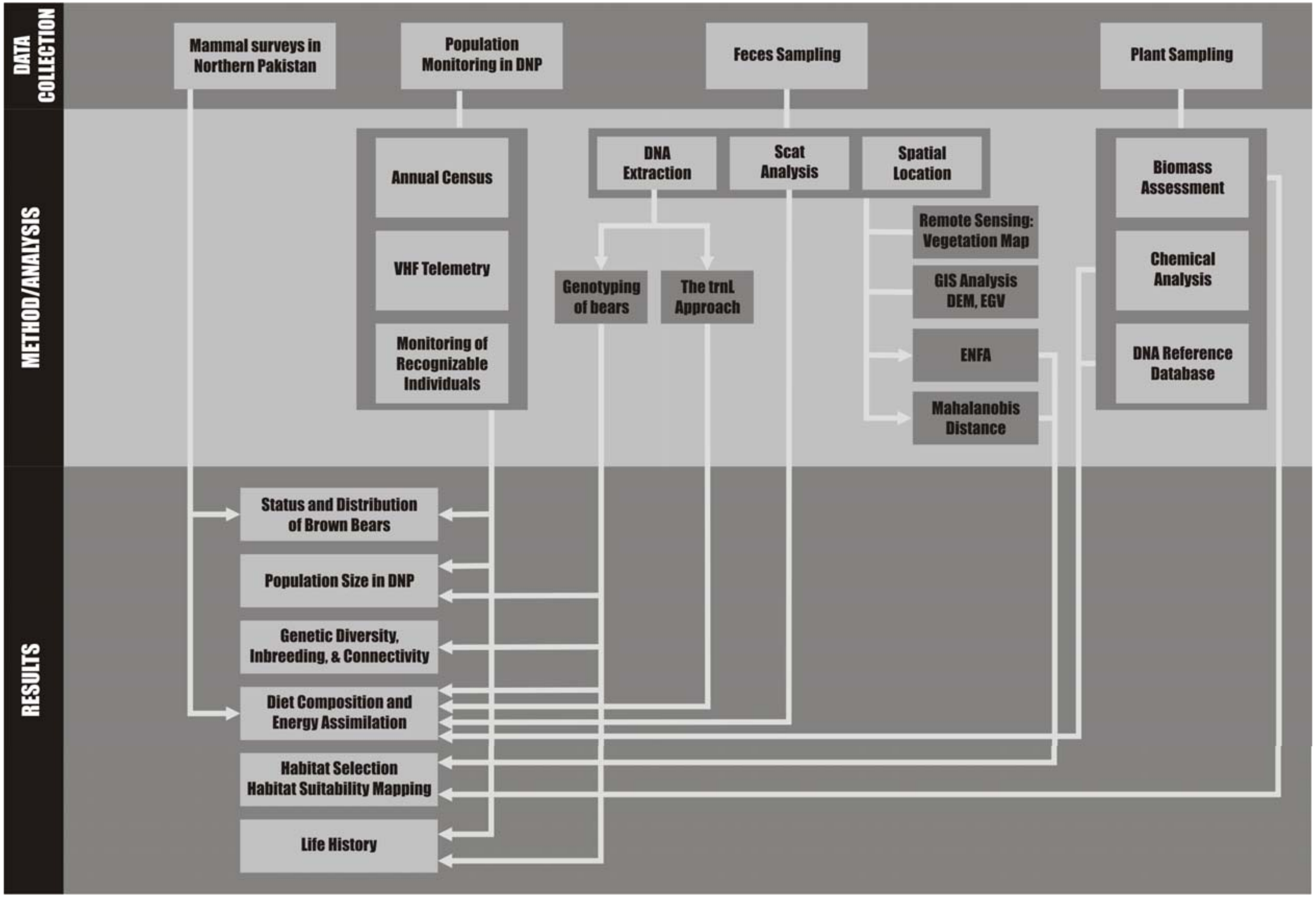

Figure 2: Methodological framework of the study

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 used 136 scat samples in genetic analyses, 334 scats in diet analyses, and locations of 450 scats in habitat analyses. We also collected 112 plant specimens (Appendix A) from Deosai for developing a reference database to be used in the *trn*L approach for the diet study (see details below). These plants were identified by taxonomists from the University of Karachi, Karachi, Pakistan Museum of Natural History, Islamabad, and Quaid-i-Azam University, Islamabad.

3.3 Status and Distribution

We conducted field surveys, interviews and consulted published and unpublished literature. Field surveys were conducted in Neelam and Gurez valleys of Azad Jammu and Kashmir, in the Northern Areas of Pakistan, and eastern part of the NWFP Province. We interviewed people in local communities, mountain nomads (*gujjars*), field staff of wildlife or forest departments, tourist operators (particularly for glacier areas), wildlife biologists, and relevant institutions and organizations.

DNP was surveyed every year (1993-2006) during 10-15 days in late September or early October to obtain a population census. The recognizable bears monitored during the summer season (see details below) helped us avoid double counting and increased the reliability of the census. We also estimated population size from the genetic data (see below) by rarefaction indices, using equations developed by Kohn et al. (1999) and Eggert et al. (2003). These methods calculate the population size as the asymptote of the relationship between the cumulative number of unique genotypes and the number of samples typed.

We estimated the finite rate of increase (λ) from annual censuses of the Deosai population, with λ as the ratio of numbers in two successive years (Caughley 1977). The λ was calculated by the exponential rate of increase, β, which was estimated by regressing population size (ln N) on year.

3.4 Genetic Diversity

DNA extractions were performed using the Qiamp DNA Stool Kit (Qiagen, Netherlands). For individual identification, the extracted DNA was amplified using the six microsatellite primers; Mu23, Mu50, Mu51, Mu59, G10J and G10H. For sex identification, we used the sex-primers described in Bellemain and Taberlet (2004). To estimate population genetics parameters and relatedness, we amplified the following 12 additional microsatellites: G1A, G1D, G10B, G10C, G10L, G10P, G10X, G10O (Paetkau et al. 1995; Paetkau and Strobeck 1994) and Mu05, Mu10, Mu15, Mu61 (Taberlet et al. 1997), using a modified protocol from Waits et al. (2000). A quality index (Miquel et al. 2006) was calculated for each sample and locus. The loci G10P, Mu05, and Mu61 were discarded from the analysis because of their low quality-indices (below 0.6). Finally, genotypes were obtained based on 15 loci. The multilocus gentotypes allowed us to determine the gene flow between the brown bear population in DNP and neighboring areas.

Using the software GIMLET version 1.3.1 (Valière 2002), we computed the probability of identity. We used a Bayesian approach to detect and date a potential bottleneck in the Deosai bear population. This method is implemented in the MSVAR program (Beaumont 1999), available at http://www.rubic.rdg.ac.uk/~mab.

Based on the 15 loci genotypes, we ran population genetic analyses using the softwares GENEPOP version 3.4 (Raymond and Rousset 1995) and GENETIX version 4.02 (Belkhir et al. 1996-2004). Nuclear genetic diversity was measured as the number of alleles per locus (A), the observed heterozygosity (Ho), as well as Nei's unbiased expected heterozygosity (He) (Nei 1978). Deviations from Hardy-Weinberg equilibrium were tested using an exact test. We calculated pairwise genetic relatedness between pairs of individuals using Wang's estimator (Wang 2002) and the software SPAGeDi version 1.0 (Hardy and Vekemans 2002).

3.5 Diet Composition and Energy Contribution

We surveyed the park area and surrounding valleys in order to estimate the biomass of ungulates and golden marmots available to brown bears. The composition of the brown bear diet was investigated by combining three methods; scat analysis, *trn*L approach, and stable-isotope analysis.

Scat analysis: Scats were soaked and washed through a 0.8-mm mesh. We selected three sub-samples from this homogenized mixture, and 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. To adjust for differential digestibility of diet items, we applied Correction Factors (CF) proposed by Hewitt and Robbins (1996). We estimated the energy contribution of each component of diet, by multiplying adjusted volumes by their respective estimated digestible energy values. For animal matter we used digestible energy values reported in Pritchard and Robbins (1990). For plants we collected fresh samples of 20 plant species (9 graminoid, 10 forbs and 1 shrub) during early August 2006. These plants were weighted when fresh, air dried, and stored in paper envelopes. The chemical analysis was conducted at the Animal Science Institute, National Agricultural Research Council, Islamabad. The following parameters were determined using methods described in AOAC (1984): dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen free extract (NFE), and total digestible nutrients (TDN). The digestible energy (DE) was calculated as: DE (Mcal/kg) = 0.0365 x TDN%+0.172 (Fonnesbeck et al. 1967; Fonnesbeck 1968).

Genetic analysis (the *trn*L approach): The genetic analysis was carried out in four main steps (Paper IV and V); 1) total DNA was extracted from about 10 mg of a feces sample with the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). 2) Each sample was amplified with primers *g* and *h* (Taberlet et al. 2007), 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.. 3) Large-scale pyrosequencing was carried out on the 454 sequencing system (Roche, Basel, Switzerland) following the manufacturer's instructions, and using the GS 20. 4) To determine bear diet, the sequences were first compared to the reference database (developed from DNP plants, Appendix A) and then, if no match was found, to public databases using the MEGABLAST algorithm (Zhang et al. 2000). We plotted the frequencies of identified families and classified them as regular (≥10% occurrence) and occasional diet items (<10% occurrence) for brown bears. Families with >50% frequency were considered as preferred plant food for bears.

Stable-isotope analysis: The fundamental concept in stable-isotope analysis is that the stable-isotope ratios in a consumer's tissues are related to its diet (Hobson et al. 2000), therefore measurement of animal tissue reveals its ingested diet. Hair samples from six radio-collared brown bears (Paper III) were analyzed for ^{15}N and ^{13}C by G.V. Hilderbrand, Washington State University, USA by the method described in Hilderbrand et al. (1996). The interpretation of stable isotope values in terms of meat content in the diet requires knowledge of stable-isotope values in the food items (plants and animals). As we do not have such values for DNP, we used isotope measurements of food items reported by Hobson et al. (2000) from British Columbia, Canada ($\delta^{15}N$ -2.1 and 3.3 for plant and animal food respectively), and calculated dietary meat of the brown bear population using equation no. 5 in Hobson et al. (2000).

3.6 Habitat Selection

We used the 28 July 1998 LANDSAT Thematic Mapper (TM) satellite image to classify DNP into six vegetation based habitat classes; marshy, grassy, stony, rocky, water and snow (Fig 3). A habitat-specific index of forage production was obtained by sampling standing crop (Soest 1994; Vallentine 1990). A digital elevation model of DNP was prepared using elevation data from the Shuttle Radar Topography Mission (SRTM) (http://www2.jpl.nasa.gov/srtm/) and topographical sheets of the Survey of Pakistan (Fig. 4). Streams were digitized from the 30 September 2001 LANDSAT image, and roads were digitized from topographical maps of the Survey of Pakistan. Locations of camps belonging to nomad and local livestock herders and seasonal hotels were recorded with a GPS receiver. An index of grazing impact was obtained from the proportion of plants grazed in quadrats.

We used the Ecological Niche Factor Analysis (ENFA) (Hirzel et al. 2002) to investigate habitat preferences of the brown bear. Eleven ecogeographical variables (described above, details in Paper VI) were used as explanatory variables in ENFA and locations of brown bear feces were used as indicators of areas used by bears. The ENFA extracts one axis of marginality and several axes of specialization. The marginality axis measures the difference between the conditions used on average by the species and the mean available habitat, whereas the specialization measures the width of the niche within available habitat. The Mahalanobis distance statistic (Clark et al. 1993) was used to compute a habitat suitability map. In order to evaluate validity of the habitat suitability map, we computed a curve of the ratio of expected-to-predicted frequencies of evaluations points (Hirzel et al. 2006).

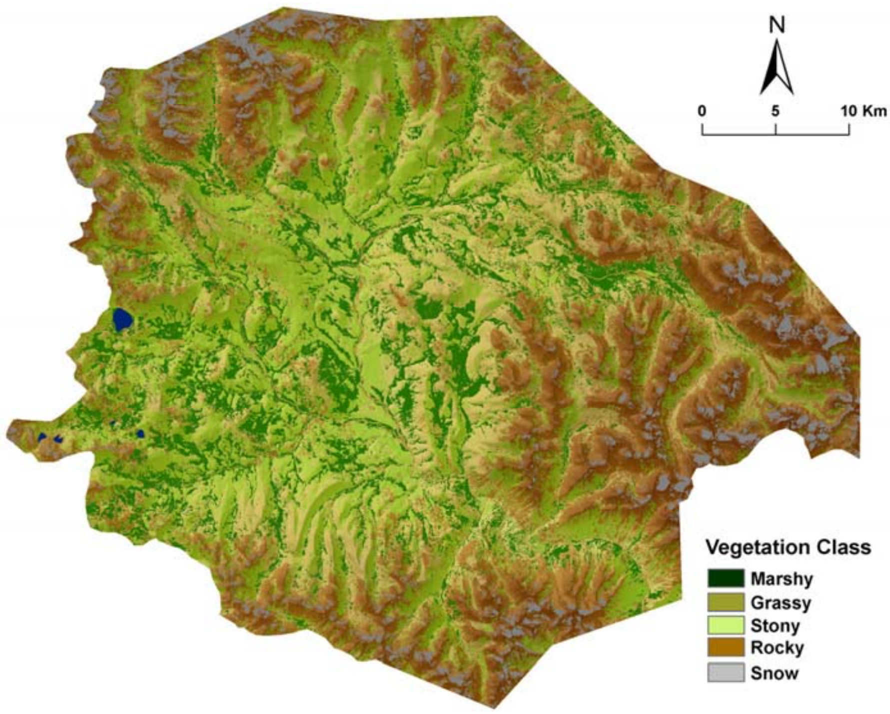

Figure 3: Vegetation map of Deosai National Park, Pakistan, based on LANDSAT Thematic Mapper

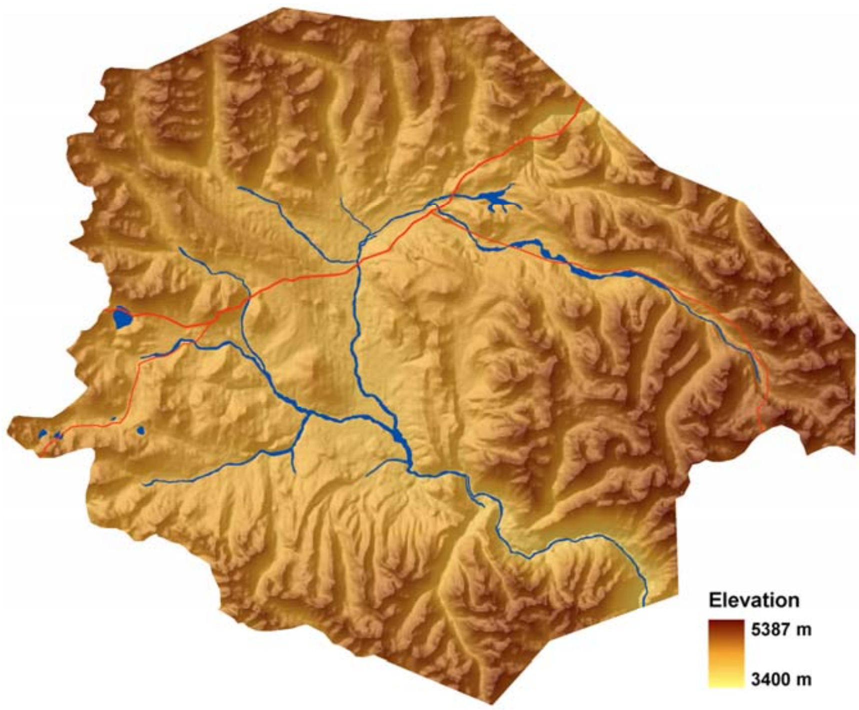

Figure 4: A Digital Elevation Model of Deosai National Park, Pakistan, developed from the SRTM Data

3.7 Life History

The park staff of the Himalayan Wildlife Foundation (HWF) operated a summer field camp in DNP from 1993-2006. The staff observed individual bears regularly and documented their movements and behavior. The following factors helped in individual recognition: 1) Color variation; in DNP four pelage colors were identifiable; blonde, silvertip, light brown and dark brown (Fig. 5). 2) White patches; many individuals had characteristic white patches, which differed in size and shape. 3) Size; brown bears are sexually size dimorphic (Schwartz et al. 2003), adult females in Deosai have a mass of 60-80 kg and adult males 120-150 kg. 4) Radiotelemetry; the 7 radio-collared adults comprised about 40% of the adult population at that time. This increased the reliability of the observational study. 5) Genetic analysis; a genetic analysis of the population (Paper II) gave a population estimate similar to the results of the field census, verified maternal relationships among individuals that were assumed from field observations, and also verified patterns of individuals' distributions as observed in the field.

This study particularly targeted females with young, which allowed documentation of the females' reproductive activity and survival of young. We used method of Garshelis et al. (1998) to calculate age of first reproduction, and extended this method to estimate litter interval and length of family association. We calculated mean litter size using all litters observed after den emergence. We used two methods to calculate reproductive rate (young born/ year/ reproducing adult female): 1) by dividing the mean litter size by the mean litter interval, and 2) from the reproductive history of 6 females that provided 11 complete birth intervals.

We estimated the minimum mortality rate by dividing the recorded deaths by the number of bears observed. Some females and associated young disappeared from the study area during the winter, and we were not sure about the fate of the associated young. We therefore reported mortality in a range of minimum (based on known mortalities) and maximum (by including undocumented loss). We calculated intrinsic growth (based on reproduction) for the best and worse case scenarios, using minimum and maximum mortality rates, respectively, using the deterministic Leslie matrix (Leslie 1945, 1948) and the Vortex Program (Lacy et al. 2006).

Figure 5: Photographs of brown bears from Deosai National Park, Pakistan, showing pelage color variation among individuals.

4. RESULTS

4.1 Status and Distribution (Papers I, II, III)

Today approximately 150–200 bears may survive in seven populations in northern Pakistan. All of these populations are probably declining, except for the population in DNP. In DNP we counted 19 individuals during 1993, which increased to 43 individuals towards end of the study. Averaged over the study period, there were 41% adults, 8% subadults and 18% young (up to 4 years of age) in the population. The adult sex ratio remained quite equal, except for recent years when it became male biased. Among the 11 cubs that successfully grew to adults during the study period, the female-to-male ratio was 6:5.

From the genetic analysis of fecal samples in 2004, 28 individual genotypes were obtained (16 males, 10 females and 2 individuals of unknown sex). The probability of identity for unrelated individuals was 1.881e-05 and 1.206e-02 for related individuals (siblings), thus we could identify each individual reliably. The Kohn's estimate yielded a population size of 47 bears (95% CI: 33-102), whereas the Eggert's estimate gave an estimate of 32 bears (95% CI: 28-58). The finite growth (λ) of the population in DNP was estimated from the annual censuses at 1.05 (95%CI: 1.03, 1.07).

4.2 Genetic Diversity (Paper II)

The number of alleles per locus among the 28 individual genotypes ranged from 2 to 7, with an average of 3.87 ± 1.36 (Appendix B provides consensus genotypes). The mean observed heterozygosity was 0.557, a value not significantly different from the unbiased expected heterozygosity (0.548). Global tests showed that the population is at Hardy-Weinberg equilibrium, although three loci (G10L, G10O, Mu10) had a significant deficiency in heterozygocity at the $p<0.05$ level. The overall multilocus Fis value was -0.016. The average pairwise relatedness in the Deosai bear population was 0.0265 ± 0.292 (SE), which was not significantly different from that for the Scandinavian populations.

4.3 Diet Composition (Papers IV, V)

About 70% of the scats were composed of only plant residues. Graminoids (grasses and sedges) had the highest frequency (93%), and constituted the bulk (85%) of the volume of the scat residues. The diet category with the second highest frequency was forbs, at 52% (presence verified by stems and inflorescences only). The volume of animal residues was only 4%, with rodents constituting most (88%) of it. With the *trn*L approach, we found a total of 57 plant taxa in the bear feces, belonging to 50 genera and 29 families. The regular plant diet (≥ 10% occurrence) of brown bears was comprised of only 8 families; Poaceae, Polygonaceae, Cyperaceae, Apiaceae, Asteraceae, Caryophyllaceae, Lamiaceae, and Rubiaceae. The first four families constituted the preferred diet, with more than 50% occurrence (Fig. 6).

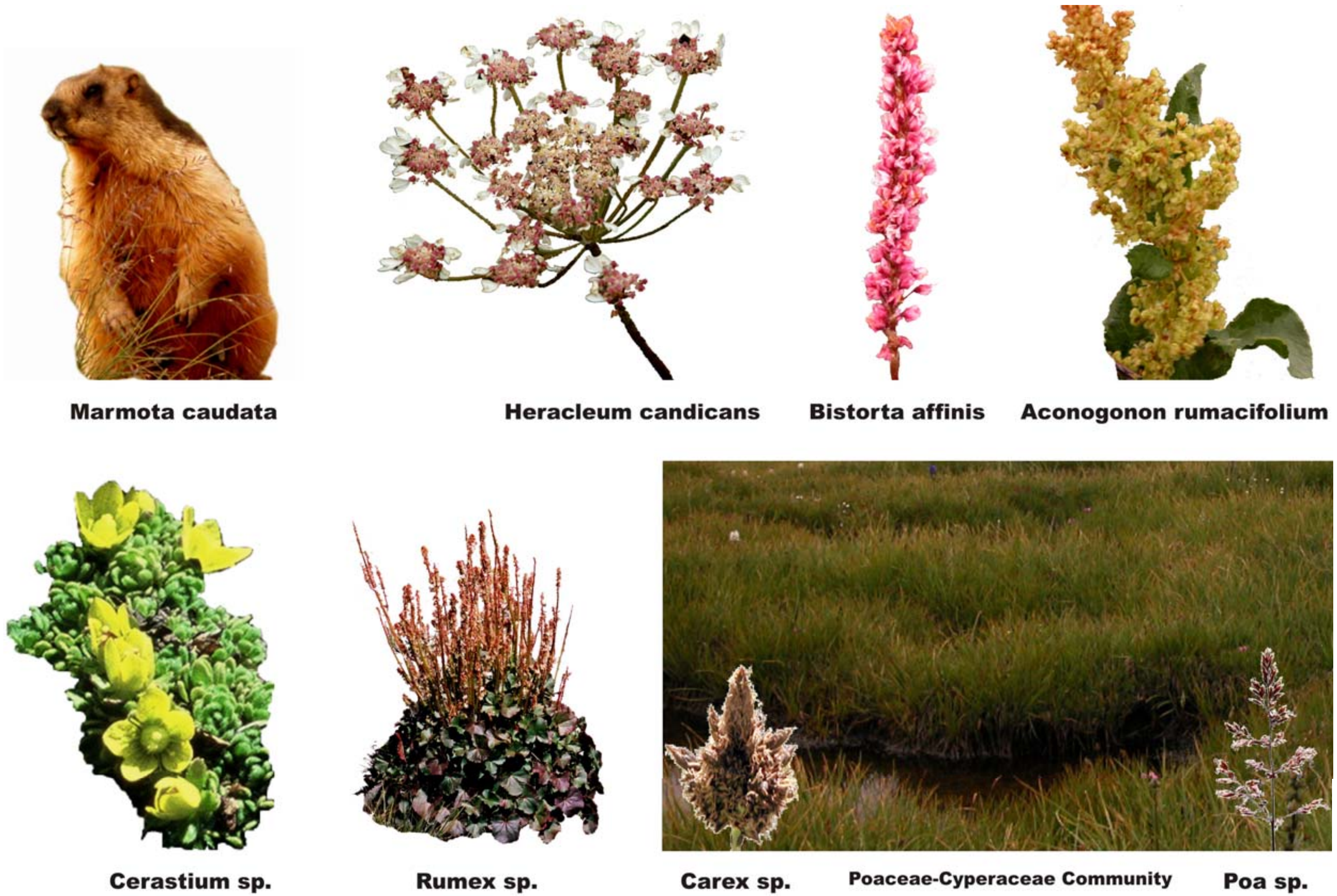

Figure 6: Common food species of brown bears in Deosai National Park, Pakistan.

The average values of stable-isotope from hair samples were 3.23 (SD: 1.127) and -23.6 (SD: 0.303) for $\delta^{15}N$ and $\delta^{13}C$, respectively (Appendix C). Assuming similar levels of isotopic values of plant and animal food in DNP as that reported by Hobson et al. (2000), the average contribution of animal matter in diet of brown bears was estimated at 9.5%, ranging from 0-27% in individuals. One subadult male was probably not consuming animal food at all. Excluding this individual gave an average meat consumption of 18.5% for the remaining of five individuals. The amount of dietary animal matter was positively related to the body mass of individuals (r= 0.59).

The average values of crude protein, crude fat and nitrogen free extract (carbohydrates) for nine graminoids were 11.6 (SD: 3.01), 3.8 (SD: 2.46) and 50.5% (SD: 4.27) dry matter, respectively (Appendix D). For forbs, the average values of these parameters were 12.7 (SD: 3.62), 3.3 (1.35) and 48.0% (SD: 9.29). The digestible energy (kj/g) was estimated at 11.8 for graminoids, 11.2 for forbs, and 12.2 for shrubs. In the diet of brown bears, 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. 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.

Males were more carnivorous than females, and they also ate higher proportions of three plant species; *Bistorta affinis*, *Carex diluta*, and *Carex* sp. Four habitats of DNP were homogenous with respect to the diet of brown bears, but diet differed significantly between the park and the surrounding valleys. In the valleys, the diet consisted predominantly of graminoids and crops, whereas the park provided more nutritious and diverse food.

4.4 Habitat Selection (Paper VI)

Bear habitat use differed significantly from random (Fig. 7), as indicated by randomization tests carried out on marginality and the first axis of specialization ($P < 0.001$, for both tests). Bears strongly avoided higher elevations and steeper slopes, and showed a higher preference for more productive parts of the park (marshy, grassy, and stony vegetation types). Brown bears tolerated human structures, such as roads and camps, but strongly avoided grazing areas with high livestock density. DNP had a range of poor to excellent habitat for brown bears. About 49% of the area was classified as poor habitat, 39% was suitable, and 12% of the area constituted high quality habitat. The habitat suitability map generally followed the biomass productivity patterns of the park. It identified the central part as suitable, and classified half of the park, mainly peripheral areas, as not suitable for brown bears. A validity test indicated good predictive power for the suitability map (Boyce Index; r: 0.98, $P < 0.01$).

4.5 Life History (Paper III)

We calculated the mean age of reproduction as 8.25 years (range: 7-10), and the mean litter interval as 5.7 years (range: 4-8). Litters consisted of 1 or 2 cubs, and averaged 1.33. The proportion of two-cub litters was 0.3. Both methods produced similar estimates of reproductive rate (natality), at 0.23 (SD: 0.066) cubs per adult reproducing female per year.

The survival estimates for the cubs-of-the-year (0.800-0.965), yearling (0.848-1.00), and ≥ 2 age group (0.923-0.976) were all within ranges, without point estimates, because we could not resolve undocumented loss in the population. The estimates of intrinsic growth (based on reproduction) by the Leslie matrix and Vortex methods were similar but lower than the observed growth. The intrinsic population growth rates estimated under best- and worst-case scenarios considering survival rates were 0.965 and 1.030, respectively, indicating uncertainty in the intrinsic population growth. However the population would be intrinsically stable only if at least half of the undocumented loss actually survived (λ: 0.997 at 50% survival of undocumented loss).

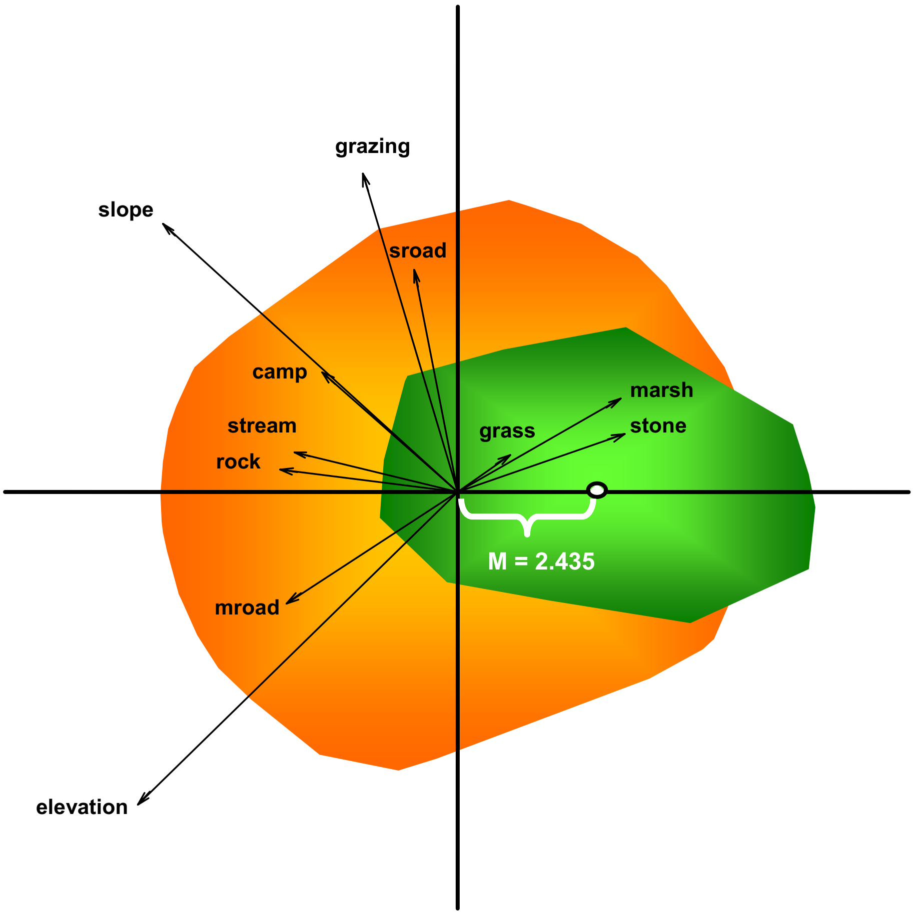

Figure 7: Biplot of the Ecological Niche Factor Analysis of brown bear habitat in Deosai National Park, Northern Areas, Pakistan. The brown area represents the available habitat and the green area corresponds to the ecological niche of the brown bear (used area). The plane consists of marginality on the X axis and the first specialization on the Y axis. Ecogeographical variables are projected by arrows. The marginality (M) factor measures the difference between the conditions used on average by the species and the mean available habitat.

5. DISCUSSION

5.1 Status and Distribution of Brown Bears in Pakistan

The Himalayan brown bear historically occupied the western Himalayas, the Karakoram, the Hindu Kush, the Pamir, the western Kunlun Shan, and the Tian Shan ranges in southern Asia. In Pakistan the subspecies ranged over approximately 150,000 km^2 in the northern part of the country. However it has been extirpated from the southern part of its historical range in Pakistan, and remaining population are no longer contagious (Fig. 8). The brown bears' range in Pakistan falls under three administrative divisions (Azad Jammu and Kashmir, Northern Areas of Pakistan, North West Frontier Province), and, as the wildlife management is a provincial subject in Pakistan, these administrative divisions have three different governing legislations. Bears are legally protected, however, and recently designated as critically endangered in IUCN's Red List of Mammals of Pakistan.

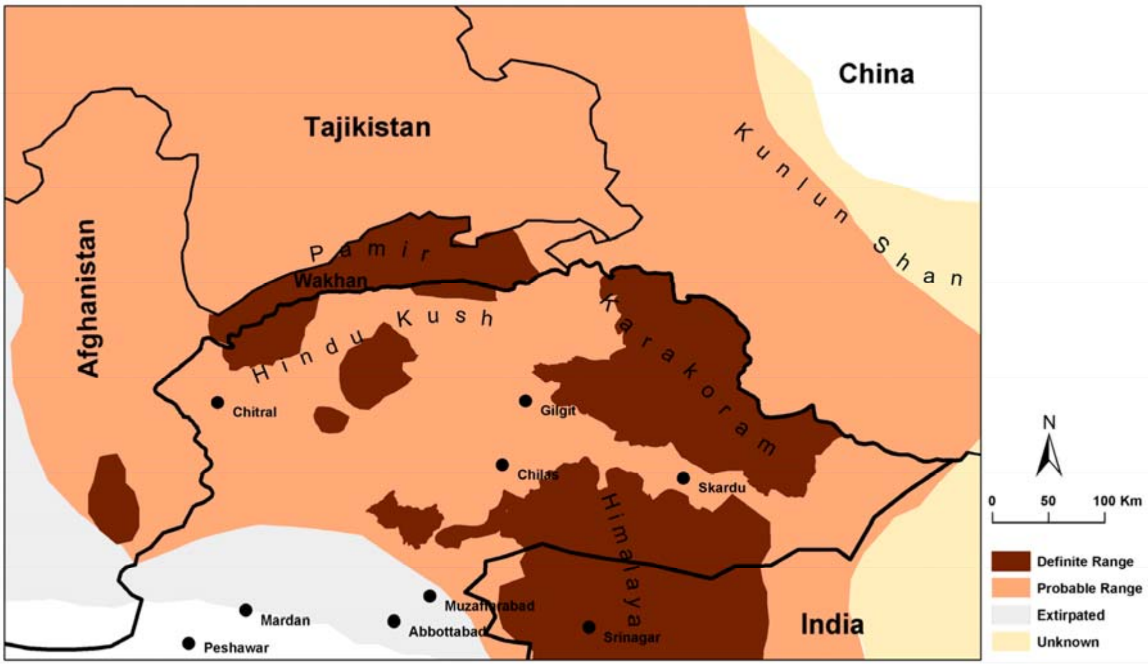

Figure 8: Present distribution of brown bears in Pakistan and neighboring countries. (Prepared in collaboration with IUCN SSC Bear Specialist Group)

In Deosai National Park, the population size estimates provided by the two rarefaction indices were in the same order of magnitude as the numbers derived from the field censuses, which gives us confidence that those results are realistic. The Eggert method seemed to underestimate the population size, whereas Kohn's method seemed to be more realistic, although the upper limit of the confidence intervals (102) seemed to be an overestimate. We conclude that approximately 40-50 bears were present in the park in 2004. Four individuals in our genetic dataset showed private alleles at two different loci, suggesting that they could be migrants (or descendants from migrants) from outside of the study area. Field observations support this hypothesis (Paper I). Brown bears also exist in the Minimerg and Astore valleys, which are adjacent to Deosai National Park. Movements of bears have been observed between these areas during recent surveys, and the Deosai population may have interchange not only with bears in these valleys, but also with the bear populations in the Neelam Valley and in Indian Kashmir through these valleys.

Considering the geomorphology of the area, evidence collected during field surveys, and genetic results, we conclude that the Pakistani populations of brown bears exhibit regional connectivity primarily through three corridors: the Himalayan population is connected to the populations in Zanskar and Ladakh ranges in India, the Hindu Kush population is connected to bears in the Tian Shan Range through the Pamir population in the Wakhan Corridor (Afghanistan) and Central Asia, and the Karakoram population may have connectivity with the Kunlun Shan in China (Fig 8, Paper I).

5.2 Genetic Diversity

The population genetics analyses revealed that the level of nuclear genetic diversity of the Deosai population was globally lower than brown bear populations that are considered to have a good conservation status, such as in Scandinavia or North America (Paper II). However, the population is in Hardy-Weinberg equilibrium and its level of relatedness was similar to that in the Scandinavian brown bear population. Therefore, the Deosai bear population does not appear to be at immediate risk of inbreeding depression. Its level of genetic diversity is comparable to the brown bear population in the Yellowstone area, USA. Lacy (1987) suggested that even a low frequency of migration between populations minimizes loss of genetic diversity associated with small population size. We believe that the moderate level of genetic diversity observed in DNP has been maintained by gene flow with adjacent populations in Pakistan and India.

We found a genetic signature of the population bottleneck. The results from the population analysis using the program MSVAR suggested that a decline in the Deosai population occurred approximately 80-100 generations ago. The ancestral population (before the decline; N1) was estimated at 10,000-12,500 individuals. This estimate seems realistic considering an approximate area of 200,000 km² of bear distribution range in northern Pakistan and Kashmir, which gives a density of about 55 bears per 1000 km². The historic phase of glaciations in High Asia (Kuhle 1997; Meiners 1997) may have acted as a proximal cause of this decline, destroying part of the population and fragmenting the rest. The influence of a growing human population, political unrest due to presence of the Tibetan army in the area and its clashes with local people and China (Sheikh, 1998; Rashid S, personal communication), and the spread of firearms in the late 19th century probably contributed further to the population decline and did not allow bears to disperse in a natural way.

5.3 Resource Selection

The *trn*L approach, stable isotope analysis, and classical scat analysis are complementary techniques, and together can provide a comprehensive understanding of feeding ecology of an omnivore species like brown bear. The *trn*L approach provided a more accurate description 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 stable-isotope analysis does not provide details on composition of the diet, but is useful in determining amount of the animal matter in the diet.

The brown bear diet was quite diverse in DNP, represented by 57 plant species, insects, ungulates and several rodent species. However the adjusted diet content indicated that only graminoids (represented by sedges and grasses) and golden marmots comprised the bulk of the diet, and provided 81% digestible energy (Paper V). Looking at plant and animal resources separately, we found consumption in accordance with availability. Graminoids are the most abundant, concentrated and consistent source of forage in DNP, and they were the dominant component of bear diet. Likewise golden marmots comprised the major mammalian biomass in the park, and were also the main protein source for bears. The stable-isotope analysis also agreed with the results of scat analysis, but indicated a lower amount of animal matter, which could be due to; 1) small sample size, particularly large males, which are more carnivorous (Paper V), were not represented in isotope samples, 2) calculations were based on isotope values of food plant and animal matter from another geographical location, and isotope signatures vary geographically (Chamberlain et al. 1996; Garten 1993). Soil ^{15}N is known to become depleted with altitude (Mariotti et al. 1980) which can be reflected in local food webs (Gröcke et al. 1997). Isotopic measurements from vegetation and animal prey from DNP, and also from more individuals, would be required to appropriately interpret the results of the stable isotope analysis.

Multivariate methods, such as ENFA or Mahalanobis distances, allow the inclusion of several variables (elevation, slope, human disturbance, vegetation types) simultaneously in analyses and therefore allow a more comprehensive understanding of habitat selection. In contrast to the diet, habitat selection by brown bears differed significantly from the mean of available conditions (Fig. 7). Habitat selection by brown bears in DNP was related primarily to biomass production, and marshy vegetation was the most selected habitat, which is consistent with the finding of diet analysis (because graminoids dominate the plant community in marshy habitats, Paper VI). Moreover, the abundance of golden marmots, which is the main protein source for brown bears, was also highest in marshy areas (1.4 times higher density than in grassy and stony vegetation, Paper V). Both diet and habitat analyses highlighted the importance of marshy areas for bears. These habitats cover only 15% of the park area, but produce half of the park's vegetation biomass. Vegetation in marshy habitats remains physiologically active, and thus nutritious, even during the late growing season, due to the availability of water (Graham 1978; Hamer and Herrero 1987). The marshy habitat, which provide a continuous, nutritious, abundant and concentrated source of forage, is therefore the key factor explaining habitat selection by brown bears.

5.4 Life History

Brown bears occupy a wide geographical range (Servheen et al. 1999), and variation in its life-history traits has been documented earlier in North American populations

(Bunnell and Tait 1981). European studies widened this spectrum further by documenting the life history of Scandinavian populations, which are the most productive in the world (Sæther et al. 1998). The reproductive parameters of 35 North American and European brown bear populations range between mean ages of first reproduction of 3-9.6 years, mean litter sizes of 1.4-2.5 cubs, mean reproductive intervals 2.4-5.8 years, and mean reproductive rates of 0.36-0.96 cubs/year/adult female (Paper III). High-latitude brown bear populations are reported to be less productive than other terrestrial and coastal brown bear populations (Ferguson and McLoughlin 2000). However we documented that the reproductive parameters of the high-elevation brown bear populations are even lower than those of the high-latitude populations. The population in the Eastern Brooks Range, Alaska, is the least productive in North America (Paper III), but is 1.8 times more productive than the Deosai population. At the other end of the spectrum, the Scandinavian population has a reproductive rate that is 4.2 times higher than the Deosai population (Paper III). Thus, by documenting the life history of high-elevation brown bears in Asia, this study has increased the known range of life-history traits in brown bears considerably. Consistent with our findings, Blumstein and Arnold (1998) reported delayed ages of reproduction and infrequent reproduction in golden marmots living in high elevations of Himalaya.

The energy allocation theory assumes that reproductive strategies are the result of an optimal allocation of surplus power (the part of the acquired energy left after satisfying metabolism) to growth and reproduction (Demetrius 1975; Ware 1980). The quantity of the surplus power depends on the available energy and the cost of maintenance in an environment (Stearns 1992). The brown bear is an opportunistic, omnivorous species, and consumes a large variety of food according to local conditions. Meat is the most nutritious food, and has a positive influence on reproductive performance in brown bears (Bunnell and Tait 1981; Reynolds and Garner 1987; Hilderbrand et al. 1999). Fruits are the second most important source of energy (Pritchard and Robbins 1990), however a mixed diet (meat and fruits) is most desirable for growth of brown bears (Robbins et al. 2007). Energy assimilation from food in 22 brown bear populations averaged 22.5 kj per gram of ingested food (Paper V). The Deosai population lacked fruits in its diet and had relatively little meat, consequently it assimilated the lowest amount of energy from its food of all brown bear populations with comparable data (35% less than the average for 22 other populations). Our results also showed that the food energy was even lower for the female brown bears, because they had relatively lower amounts of meat in their diet than males (Paper V). The very low amount of food energy and higher cost of metabolism at high altitudes (Mani 1990; Westerterp and Kayser 2006), probably contributed to the very low reproductive rates of the brown bear population in DNP. This conservative life-history strategy, however, may have selective advantages in high-altitude environments, because low fecundity increases the population's ability to persist in stochastic environments (Demetrius 1975; Murdoch 1966).

6. IMPLICATIONS FOR MANAGEMENT

It was interesting to learn that brown bears with such a low productivity still could maintain their existence in such environmental extremes, while obtaining most of their diet from grasses. However our results show that population is highly sensitive to harvesting, because of their limited intrinsic growth potential. Hunting has been a traditional practice in northern Pakistan, and is also the key threat to Himalayan brown bear populations in other parts of their range (Servheen et al. 1999). Increasing accessibility to bear habitat has increased hunting in recent decades. Bears have been hunted for sport, persecuted by villagers who feel their livestock is threatened, and also killed for commercial purposes. Hence poor growth potential makes their conservation highly challenging. Nevertheless, our study documents that these low-productive bears can be conserved by reducing human-caused mortalities, particularly of adult females.

Deosai National Park supports a growing population and the highest density of brown bears yet documented in High Asia. It should remain the focus of conservation efforts, because the future of the brown bear in Pakistan, and perhaps in the region, largely will depend on stability in this park. Current protection and monitoring must be maintained, and connectivity with neighboring populations should be improved. Managing human resource use without affecting the brown bear population has been a major management challenge in the park, and seems to have been achieved. However a large influx of livestock by nomad grazers in recent years needs urgent attention, if the brown bear population is to continue to recover. We recommend monitoring the numbers and distribution of livestock and conducting a detailed inventory of the rangeland to understand grazing dynamics in the park and to maintain sustainable stocking rates.

We documented movement of brown bears between Deosai and adjoining areas, which has important implications for conservation, through maintaining gene flow and influencing demographic processes. Because some individuals apparently have home ranges larger than the park, we recommend that protection be extended to the adjacent valleys, while allowing communities to sustain their livelihoods. We also documented that the Pakistani populations are connected to other regional populations through three corridors (Fig. 8). These movement corridors, particularly the Neelam Valley and the Pamir Range, provide ideal venues for management of brown bears on broader landscape through cross-border cooperation. A peace park around the Pamir Knot (involving Afghanistan, Tajikistan, China and Pakistan) is already under consideration, and the Neelam Valley along the Line of Control with India provides another opportunity. Such initiatives would benefit many other threatened large mammals as well, including the Asiatic black bear (*Ursus thibetanus*), common leopard (*Panthera pardus*), snow leopard (*Panthera uncia*), musk deer (*Moschus moschiferus*), Himalayan ibex, and Marco Polo sheep (*Ovis ammon polii*).

The presence of humans in occupied brown bear habitat is a reality, and the livelihood of local people is linked with it. Conservation planning based on the exclusion of people and implemented with force therefore has a very poor chance to succeed. DNP was managed on the participatory approach and the observed growth of brown bear population suggests that the park has been successful in achieving its major goal. This success adds to the growing recognition that the local communities should be integrated in planning and management of protected areas (PAs) (Dearden et al. 2005; Hiwasaki 2005). Changes to the legislative and regulatory framework of the PAs that would recognize the rights of communities and provide the framework for community participation and benefit sharing would promote the involvement of the local people. Participation of local communities in the management process not only minimizes conflicts, but also leads to efficient conservation planning.

PAs cover most of the brown bear range in Northern Pakistan. However, most of these are poorly managed, due to limited financial resources and lack of training of the management staff. Strengthening the PA system and improving its efficiency in Pakistan can prevent many endangered mammals from declining further. Carnivores as a whole are considered odious and it is usually difficult to generate support by local communities for their conservation. People always question such efforts because, unlike ungulates, carnivores do not have any meat value and pose a threat to humans and livestock. Environmental education is an important instrument to change perceptions and attitudes. Launching education and awareness initiatives that cater to local communities, staff of the PAs, visitors, and the general public can bridge the knowledge gap and be vital to achieving synergy in conservation efforts.

7. RESEARCH PERSPECTIVES

This study documents, for the first time, the ecological requirements, life history and genetic structure of the Himalayan brown bear. Therefore it provides the basic information required for the population and habit management of brown bears in Himalaya. There are, however, several questions to answer that are beyond the limits of our data or have arisen based on our results. We list here some of the questions that need to be pursued during future research.

We documented that the population growth in DNP was the product of reproduction and immigration (Paper III). We estimated reproductive parameters and survival from observations of recognizable individuals. Some individuals were lost from contact, due to either death, emigration, or large-scale movement, but we could not resolve their fate. In order to account for this undocumented loss to the population, we reported survival and consequently intrinsic growth rate in a range. These estimates did not allow us to adequately interpret the contribution of reproduction to the observed population growth. The visual monitoring was useful as long as the bears remained in the open plateau of the study area. To monitor individuals over larger distances in the highly rugged terrain of Himalaya and resolve such questions as long-term movements and monitoring, an advance technique such as GPS telemetry would be required. With the use of GPS telemetry, or combining it with conservation genetics techniques, we could answer many important questions, for example; 1) Determine age-specific estimates of survival and the nature and magnitude of threats to bears beyond the boundary of the national park. 2) Patterns of bear movements in the broader landscape, particularly to answer whether individuals are emigrating/immigrating or whether they just have large-scale movements. 3) Complete home ranges and resource selection within home ranges. Habitat and diet analysis during present study was limited to the park area. Many individuals had home ranges larger than the park, and it is therefore important to understand which resources they utilize over the course of the year. 4) Subadult bears comprised a substantial part of the undocumented loss of the population (Paper III), which might be due to dispersal. We should investigate dispersal and other aspects of social organization.

The distribution range of the Himalayan brown bear encompasses diverse habitats, from scrub land to coniferous forests and alpine meadows. We documented their habitat selection in DNP, which is an alpine habitat. Alpine meadows, particularly marshy areas, should therefore be recognized as their preferred habitat throughout their range. However their habitat requirements in other landscapes (e.g., forested areas) are likely to be different, and need to be investigated. We recommend surveys and sample collection from the rest of their range in Pakistan, and preferably throughout their range in High Asia through collaborative arrangements. This will give a comprehensive understanding of resource selection, and also allow the computing of habitat suitability maps for their entire range, which would serve as an effective management tool on regional scale. Genetic samples from the entire range of Himalayan brown bears would allow delineating populations and investigating patterns of gene flow.

In Deosai National Park, the park staff provided a cost-effective and efficient means of primary data collection. They should continue monitoring the population and habitat of the brown bears. The most important activities are annual censuses of the brown bear population, documentation of natural and human-caused mortalities, livestock numbers, and tourism activity. Following recognizable individuals has been an important component of the monitoring in the park, which contributed to reliable estimates of the population size every year. The HWF staff remained associated with the brown bear monitoring for a long time, and their experiences and ability to recognize individuals enhanced the quality of the data. Recently the HWF handed over most of the park responsibilities, including monitoring, to the Northern Areas Forest and Wildlife Department (NAWD). Most of the NAWD staff is new and inexperienced. Brown bear numbers have also increased; therefore following unmarked individuals visually will be increasingly difficult in the future. Instead we recommend counting females with cubs-of-the year for estimating population size and monitoring change in the population (Knight et al. 1995; Keating et al. 2002; Harris et al. 2007; Ordiz et al. 2007). This method would allow the park staff to evaluate their annual counts of bears in the park. The field estimates should also be evaluated with a periodic DNA-based census (e.g., every 10 years) of the populations using fecal samples. From the amplification success of this study, we recommend that fecal samples older than one week not be collected, in order to optimize the cost and benefit of the genetic analyses. Increasing the size and range of fecal sampling would not only allow a more precise estimate of the population size, but also give a better estimate of gene flow.

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The journey of my PhD project started when the International Bear Association accepted my very late submitted abstract for the 14th International Conference in Steinkjer, Norway in 2002. Prof. Jon Swenson invited me for a preconference expedition to the study area of the Scandinavian Brown Bear Research Project (SBBRP) in Sweden, and received me at the Gardermoen Airport. I enjoyed that expedition even more than the conference. This was my first visit to Europe and I found Jon to be the most friendly person in that crowd. This encouraged me to discuss my research interests during a lunch break. He was brave enough, I think, to accept a student from Pakistan, knowing that I may cost him lot more in terms of energy and resources than usual European students. It is difficult to put in words the excellent supervision he provided me. Apart from his great contribution in developing my research skills, he has been a good friend and made my time enjoyable in Norway.

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APPENDIX A REFERENCE DATABASE OF PLANT SPECIES FROM DEOSAI NATIONAL PARK, USED IN GENETIC ANALYSIS OF THE BROWN BEAR DIET

No.	Family Name	Species Name
1	Alliaceae	*Allium carolinianum*
2	Alliaceae	*Allium fedtschenkoanum*
3	Alliaceae	*Allium himalayense*
4	Boraginaceae	*Myosotis alpestris*
5	Boraginaceae	*Myosotis* sp.
6	Brassicaceae	*Brassica* sp.
7	Brassicaceae	*Chorispora sabulosa*
8	Brassicaceae	*Draba oreades*
9	Brassicaceae	*Thlaspi andersonii*
10	Brassicaceae	Unknown Species
11	Caryophyllaceae	*Cerastium cerastoides*
12	Caryophyllaceae	*Cerastium pusillum*
13	Caryophyllaceae	*Holosteum umbellatum*
14	Caryophyllaceae	*Silene tenuis*
15	Compositae	*Anaphalis nepalensis*
16	Compositae	*Aster falconeri*
17	Compositae	*Cremanthodium decaisnei*
18	Compositae	*Cremanthodium ellissi*
19	Compositae	*Hippolytia dolichophylla*
20	Compositae	*Jurania himalaica*
21	Compositae	*Lactuca lessertiana*
22	Compositae	*Leontopodium brachyactis*
23	Compositae	*Saussurea atkinsonii*
24	Compositae	*Saussurea falconeri*
25	Compositae	*Saussurea obvallata*
26	Compositae	*Senecio analogus*
27	Compositae	*Seriphidium leucotrichum*
28	Compositae	*Taraxacum dissectum*
29	Compositae	*Taraxacum officinale*
30	Crassulaceae	*Hylotelephium ewersii*

…Continued, Appendix A

No.	Family Name	Species Name
31	Crassulaceae	*Rhodiola heterodonta*
32	Crassulaceae	*Rosularia alpestris*
33	Crassulaceae	*Rhodiola adriatica*
34	Crassulaceae	*Rhodiola quadrifida*
35	Crassulaceae	*Rhodiola* sp.
36	Cyperaceae	*Carex diluta*
37	Cyperaceae	*Carex orbicularis*
38	Cyperaceae	*Carex* sp.
39	Cyperaceae	*Carex canescens*
40	Cyperaceae	*Carex* sp.
41	Cyperaceae	*Carex dioica*
42	Ephedraceae	*Ephedra gerardiana*
43	Fumariaceae	*Corydalis falconeri*
44	Gentianaceae	*Gentiana* sp.
45	Gentianaceae	*Gentianodes eumarginata*
46	Gentianaceae	*Gentianodes tianschanica*
47	Gentianaceae	*Gentianopsis paludosa*
48	Gentianaceae	*Sewertia* sp.
49	Geraniaceae	*Geranium pratens*
50	Labiatae	*Dracocephalum nutans*
51	Labiatae	*Nepeta linearis*
52	Labiatae	*Thymus linearis*
53	Onagraceae	*Epilobium angustifolium*
54	Onagraceae	*Epilobium latifolium* L. subsp*. latifolium*
55	Papavaraceae	*Papaver nudicaule*
56	Papilionaceae	*Astragalus rhizanthus*
57	Papilionaceae	*Oxytropis cachemiriana*
58	Poaceae	*Agrostis vinealis*
59	Poaceae	*Elymus longi-aristatus*
60	Poaceae	*Elymus nutans*
61	Poaceae	*Koeleria macrantha*
62	Poaceae	*Phleum alpinum*
63	Poaceae	*Piptatherum gracile*
64	Poaceae	*Poa alpina*
65	Poaceae	*Poa annua*
66	Poaceae	*Poa* sp.
67	Poaceae	*Poa supina*

…Continued, Appendix A

No.	Family Name	Species Name
68	Poaceae	*Poa*. sp.
69	Poaceae	Unknown Species
70	Polygonaceae	*Aconogonon rumicifolium*
71	Polygonaceae	*Aconogonon tortuosum*
72	Polygonaceae	*Bistorta affinis*
73	Polygonaceae	*Oxyria digyna*
74	Polygonaceae	*Oxytropis cachemiriana*
75	Polygonaceae	*Polygonum cognatum* subsp. *cognatum*
76	Polygonaceae	*Polygonum paronychioides*
77	Polygonaceae	*Polygonum pyrodiodes*
78	Polygonaceae	*Polygonum* sp.
79	Polygonaceae	*Rumex nepalensis*
80	Primulaceae	*Androsace septentrionalis*
81	Primulaceae	*Primula macrophylla* var. *macrophylla*
82	Primulaceae	*Primula schlagintweitiana*
83	Ranunculaceae	*Pulsatilla wallichiana*
84	Ranunculaceae	*Aconitum heterophyllum*
85	Ranunculaceae	*Aconitum violaceum* var. *violaceum*
86	Ranunculaceae	*Caltha alba*
87	Ranunculaceae	*Ranunculus* sp.
88	Ranunculaceae	*Ranunculus* sp.
89	Rosaceae	*Alchemilla* sp.
90	Rosaceae	*Cotoneaster affinis*
91	Rosaceae	*Potentilla argyrophylla*
92	Rosaceae	*Potentilla gelida*
93	Rubiaceae	*Artemisia dubia*
94	Rubiaceae	*Galium boreale*
95	Rubiaceae	*Galium himalayense*
96	Rubiaceae	*Galium* sp.
97	Salicaceae	*Salix caesia*
98	Salicaceae	*Salix* sp.
99	Saxifragaceae	*Saxifraga flagellaris* subsp. *crassiflagellata*
100	Saxifragaceae	*Saxifraga hirculus*
101	Scrophulariaceae	*Lagotis kunawurensis*
102	Scrophulariaceae	*Pedicularis albida*
103	Scrophulariaceae	*Pedicularis bicornuta*
104	Scrophulariaceae	Pedicularis oederi

…Continued, Appendix A

No.	Family Name	Species Name
105	Scrophulariaceae	*Pedicularis punctata*
106	Scrophulariaceae	*Veronica anagallis* var *aquatica*
107	Umbelliferae	Unknown Species
108	Umbelliferae	*Heracleum candicans*
109	Umbelliferae	*Pleurospermum hookeri* var. *thomsoni*i
110	Umbelliferae	*Pleurospermum hookeri* subsp. *tibetica*
111	Umbelliferae	Pleurospermum sp.
112	Unknown Family	Unknown Species

APPENDIX B MULTILOCUS GENOTYPES OF HIMALAYAN BROWN BEARS FROM DEOSAI NATIONAL PARK, PAKISTAN

ID	sex	Microsatellites																													
		Mu23		Mu50		Mu51		Mu59		G10Jnew		G10Hnew		G1A		G1D		G10B		G10C		G10L		G10O		G10X		Mu10		Mu15	
1	F	140	150	92	92	119	119	109	113	80	86	243	245	189	193	171	177	150	150	104	108	159	159	195	195	142	142	154	154	141	141
2	?	136	140	92	92	119	119	113	117	80	86	245	249	189	193	175	179	150	150	104	108	143	143	195	195	?	?	?	?	141	141
3	F	140	150	92	92	119	119	95	117	80	88	241	245	193	193	171	179	136	136	108	108	155	157	195	195	142	142	152	154	139	141
4	F	136	146	92	92	119	119	95	117	80	84	241	241	189	189	171	179	150	150	104	104	157	159	195	195	142	142	152	152	139	141
5	F	136	136	100	100	119	121	119	119	80	80	241	245	189	?	177	179	136	150	104	108	155	157	195	195	142	?	152	154	139	141
6	F	140	150	92	92	119	119	113	117	84	86	241	?	189	193	177	177	150	150	108	108	157	159	195	195	142	142	154	154	141	141
7	M	140	144	92	94	119	121	95	111	80	86	243	245	189	193	179	179	150	150	108	108	155	159	195	195	142	142	152	154	139	141
8	M	140	150	92	92	119	119	117	117	84	86	241	241	189	189	171	179	150	150	104	108	157	157	195	195	142	142	154	154	141	141
9	M	140	144	92	94	119	121	95	109	86	86	241	245	189	189	171	179	136	136	?	108	?	?	195	195	142	142	152	154	139	141
10	M	136	150	92	100	119	121	95	119	80	88	241	245	189	193	179	179	136	150	104	104	155	157	195	195	142	142	152	152	139	139
11	M	136	150	92	100	119	121	119	119	80	80	241	241	193	193	171	?	150	150	104	104	155	155	195	195	142	142	152	152	139	139
12	M	144	150	92	100	119	119	109	119	80	86	241	241	189	193	179	179	136	150	104	108	?	?	195	195	142	142	152	152	139	139
13	M	140	150	92	100	119	121	95	119	80	88	241	245	193	193	177	179	150	150	104	108	155	157	195	195	142	142	150	154	137	141
14	M	146	150	92	92	119	119	109	117	84	88	241	245	189	189	171	179	136	150	104	108	155	159	195	195	142	142	152	152	139	139
15	M	136	140	92	100	119	119	95	119	88	88	241	245	189	193	177	179	136	150	104	108	143	143	195	195	154	154	142	142	139	139
16	M	140	144	96	100	119	121	95	109	80	88	245	245	189	193	177	179	136	150	108	108	157	159	193	195	142	142	152	154	139	141
17	F	140	144	92	94	119	121	111	115	80	86	243	245	189	189	171	175	136	150	108	108	157	159	195	195	142	142	152	154	139	141
18	F	144	150	92	100	119	127	95	113	80	88	245	245	189	193	171	179	136	150	104	104	155	157	195	195	142	142	152	154	139	141
19	M	140	150	92	92	119	119	109	113	86	88	241	245	189	189	177	179	136	136	104	108	143	143	195	195	154	156	140	140	139	141
20	M	136	140	92	100	119	121	109	119	80	80	241	245	189	189	177	179	136	?	108	108	?	157	195	195	142	142	?	154	139	141
21	M	140	144	92	94	119	121	111	115	80	86	?	?	189	189	?	?	136	150	108	108	?	?	?	?	142	142	152	154	139	141
22	F	140	140	92	94	119	119	95	109	80	86	243	245	189	193	177	177	136	150	104	108	159	163	195	195	142	142	152	152	139	139
23	F	136	144	92	94	119	121	95	109	80	86	241	245	193	193	179	179	136	150	104	108	155	155	195	195	142	142	152	154	139	141
24	M	136	144	94	100	119	119	95	109	80	80	241	243	189	189	177	179	136	150	104	108	157	159	195	195	142	142	154	154	141	141
25	F	140	150	92	92	119	121	109	117	80	80	241	245	189	193	177	179	136	150	108	108	157	159	195	195	142	142	152	154	139	141
26	M	140	146	92	92	119	119	95	117	80	80	241	245	189	193	177	179	150	150	104	108	159	159	195	195	142	142	152	154	139	141
27	M	136	140	92	92	119	121	95	117	80	80	241	245	?	193	177	179	136	150	108	108	143	143	195	195	156	158	140	142	141	141
28	?	136	136	92	96	121	121	117	117	80	84	243	?	189	191	177	179	150	150	104	108	143	143	195	195	156	158	140	140	?	141

APPENDIX C STABLE ISOTOPE VALUES FROM HAIRS OF BROWN BEARS CAPTURED IN DEOSAI NATIONAL PARK, PAKISTAN

Sample	Sex	Weight (kg)	$\delta^{15}N$	$\delta^{13}C$	Estimated Animal Matter (%)
1	Female	68	4.0	-24.0	23.4
2	Male	55	3.5	-23.5	13.2
3	Male	55	1.1	-23.6	0.0
4	Female	79.5	3.4	-23.9	11.2
5	Female	80	4.2	-24.3	27.5
6	Female	65	3.7	-24.1	17.3

APPENDIX D NUTRITIONAL CONTENT OF PLANT SPECIES FROM DEOSAI NATIONAL PARK, PAKISTAN

Species	Forage Type	DM	Proximate composition of %DM					
		%	CP	CF	T. Ash	EE	NFE	TDN
Cyperaceae								
Carex polyphylla	Graminoid	28.9	17.27	21.9	7.76	1.74	51.33	70.83
Carex bulbeosaris	Graminoid	41	11.3	25.74	7.16	6.04	49.76	59.23
Carex alpina	Graminoid	31.9	10.49	24.52	6.23	3.99	54.77	63.15
Poaceae								
Agrostis or poa sp	Graminoid	55.3	9.51	30.19	7.41	2.28	50.61	62.14
Molinia caerulea	Graminoid	63.9	10.95	24.93	7.16	8.36	48.6	54.54
Phalaris arundinacea	Graminoid	40.8	13.12	23.89	11.68	5.98	45.33	57.79
Avena aspera	Graminoid	31	6.31	36.32	6.25	2.21	48.91	58.06
Festuca pratensis	Graminoid	26.6	11.74	21.21	5.58	2.41	59.06	68.19
Aliaceae								
Allium himolyense	Forb	23.1	9.57	20.71	4.47	2.81	62.44	69.77
Apiaceae								
Harcleum candicans	Forb	36	18.24	30.26	9.66	3.92	37.92	63.37
Astraceae								
Anaphalis nepalensis	Forb	30.25	9.27	31.67	7.19	2.74	49.13	60.86
Jurinea halaica	Forb	-	12.08	23.98	15.45	5.21	43.28	55.49
Lactuca sp.	Forb	0.12	13.3	18.27	19.72	5.78	42.93	54.34
Fabaceae								
Oxytropis cachemiriana	Forb	46.2	18.36	22.58	19.35	1.33	38.38	63.18
Polygonaceae								
Aconitum hetrophyllum	Forb	14.7	12.16	21.4	24.42	3.03	38.99	52.64
Primulaceae								
Primulla miscrophylla	Forb	22	13.8	16.56	17.28	2.39	49.97	62.5
Rubiaceae								
Galium himalayense	Forb	26.37	12.77	19.21	10.33	2.41	55.28	66.03
Saxifragaceae								
Saxifraga flagellaris	Forb	33.4	7.09	19.16	8.99	3.11	61.65	62.37
Salicaceae								
Salix caesius	Shrub	36.6	15.97	18.42	5.19	6.27	54.15	66.13

(DM: Dry Matter, CP: Crude Protein, CF: Crude Fiber, EE: Ether Extract, NFE: Nitrogen Free Extract, TDN: Total Digestible Nutrients)

Paper I

Status of the brown bear in Pakistan

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Abstract: As in the rest of their range in Southern Asia, brown bears (*Ursus arctos*) are poorly studied in Pakistan. Historically, brown bears occupied almost the entire range of the mountains of northern Pakistan, approximately 150,000 km^2. Their populations are declining and have gone extinct from some areas in the past 50 years. Brown bears are now distributed over 3 major mountain ranges and 4 intermountain highlands. The bears' range in Pakistan falls under 3 administrative divisions, and, as wildlife management is a provincial subject in Pakistan, these administrative divisions have separate governing legislation. Bears are legally protected, however, and recently designated as critically endangered in IUCN's Red List of Mammals of Pakistan. Seven populations probably persist in the Himalaya, Karakoram, and Hindu Kush ranges; the Deosai Plateau in western Himalaya hosts the only stable population. The sizes of these populations do not exceed 20 individuals, except for Deosai National Park, where 43 bears were counted in 2006. Seven national parks and many wildlife sanctuaries and game reserves, which provide legal protection to bears, have been established in the northern mountains of Pakistan. Populations in Pakistan are probably connected to those in India (to the east), China (to the north), and Afghanistan (to the west). Growing human population, expanding infrastructure, increasing number of livestock, and increasing dependency on natural resources, particularly alpine pastures, are key threats. Poaching for its commercial parts and for cubs, and growing unmanaged tourism also contribute to population decline. The population has become conservation dependent, and actions like effective management of protected areas, better management of natural resources, and environmental education need immediate attention.

Key words: brown bear, conservation, Himalaya, Pakistan, population, South Asia, *Ursus arctos*

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Worldwide, numbers and distribution of brown bears (*Ursus arctos*) have declined by about 50% during the past 100 years (Servheen 1990). The species is most endangered, but the least studied, in Asia, where small isolated populations exist mostly in remote mountainous areas (Servheen 1990, Garshelis and McLellan 2004). In Asia the brown bear populations of Turkey, Iraq, Iran, Afghanistan, Turkmenistan, Tajikistan, Uzbekistan, Kyrgyzstan, Kazakhstan, China, Mongolia, Pakistan, India, and Nepal are sparse and often isolated (Servheen 1990, Sathyakumar 1999, Servheen et al. 1999, Can and Togan 2004, Garshelis and McLellan 2004, Mishra and Fitzherbert 2004).

The Himalayan brown bear (*Ursus arctos isabellinus*) is the brown bear subspecies present in Pakistan. Brown bears are given a variety of names in the Indian subcontinent including *drenmo* in the northern areas of Pakistan (in Balti), and more specifically *spang drenmo* (spang = grass) or vegetarian bear. This is in contrast to *shai drenmo* (shai = meat), which is sometimes used for Asiatic black bears (*Ursus thibetanus*). In contrast, brown bears on the Tibetan Plateau are known to have a primarily carnivorous diet (Xu et al. 2006), with the plateau pika (*Ochotona curzoniae*) as the primary prey.

Although the brown bear is not considered to be as impressive as big cats (*Panthera* sp.), it has an impact on culture and beliefs, and many bear body parts are believed to have magical medicinal power, acknowledging the strength of the bear. In Muslim culture it is not permitted to eat carnivores (they are considered *haram*), therefore people cannot directly consume bear meat and other parts. Interestingly, people who want to gain strength from bears find a way around this restriction by feeding the fat of the bears to birds, particularly roosters, then eating those birds.

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The bear is considered an ugly, yet funny and strong animal in Pakistan, where they are still used in bear baiting events (Joseph 1997), during which a bear is tied to a stake with a short chain, and one or more bull terriers are let loose upon it. The bear usually wins, but at a great cost to itself and to the dogs. Rich feudal lords in rural areas provide the bull terriers and organize the fights, whereas *qalanders* (gypsies) train and provide the bears. Bear baiting events used to be big traditional events in Pakistan, and involved a lot of people and money. The number of baiting events has fallen with time, and there has been a strong campaign in recent years to end this cruel sport. Asiatic black bears are the major victims, while brown bears are involved in 10–15% of baiting events (B. Khanum, World Society for Protection of Animals, Islamabad, Pakistan, personal communication, 2006). Bear baiting is illegal under Prevention of Cruelty to Animals Act of 1890 (Joseph 1997), which was reinforced through a presidential order in 2001.

Monitoring of bears in the Deosai Plains (Himalayan Wildlife Foundation 1999) and interviews with people in local communities during the present surveys confirm that brown bears in Pakistan are not very aggressive animals, that they hardly ever attack people or prey on livestock, and that consequently they are not as loathed as are snow leopards (*Uncia uncia*) and wolves (*Canis lupus*). However, locals still feel that bears compete with their livestock for scant resources in alpine meadows, fear their unpredictability, and resent them for not being edible according to their traditions.

Data concerning the distribution and status of brown bears in Pakistan are scarce, patchy, and outdated, and no status report has been published in the last 5 decades. Data gathering in bear habitat is difficult due to rough terrain, poor access, harsh climatic conditions, and expensive logistics. For example, surveying glacial areas in the Karakoram Range requires trekking for weeks. This paper attempts to provide the presents status of the brown bear in Pakistan. Though the estimates provided are crude, they provide benchmark information for planning conservation interventions for this threatened carnivore.

Study area

The study area is the brown bear's distribution range in Pakistan (Fig. 1), which is distributed over 3 major administrative divisions. The Northern Areas (NAs) are administered directly by the federal government through the Ministry of Kashmir Affairs and Northern Areas, States, and Frontier Regions (MoKANA). The eastern part lies in the state of Azad Jammu and Kashmir (AJK) and is separated by a Line of Control (LOC) from Indian Kashmir. The North West Frontier Province (NWFP), commonly called *Sarhad*, covers the southern and western part of the bear range.

The area is rugged, dominated by one of the most mountainous landscapes in the world. Elevations start at 1,000 meters in the south and rise above 6,000 meters in the north. Over 60% of the area is above 3,000 meters. The landscape is characterized by 3 major mountain ranges (the Western Himalaya, the Karakoram, and the Hindu Kush), and 4 north–south oriented intermountain highlands (the Hindu Raj, the Swat Kohistan, the Indus Kohistan, and the Kaghan-Neelam) (Woods and Kalpatrick 1997). Climatic conditions vary widely in the study area, ranging from arid and semi-arid cold desert in west to the monsoon-influenced moist temperate zone towards east. Annual temperatures in valleys may vary between -10°C to 40°C. Vegetation zones are also diverse, mainly represented by alpine desert, alpine meadows and scrub, and coniferous forests. Human land use has a characteristic altitudinal pattern. Human settlements, roads, and irrigated cultivation are concentrated along the valley bottoms. Between 2000–3000 m are summer villages, with summer pastures and crops. Alpine pastures start about 3,000 m and go up to the snow line, usually at 5,000 m (Ehlers and Kreutzmann 2000, GoP and IUCN 2003).

Human density is as low as 12 people/km^2 in the NAs and rises gradually southward up to 252/km^2 in Mansehra District (Population Census Organization 2001, GoP and IUCN 2003). Despite the overall relatively low population density, the area is a mosaic of cultures and languages, with 11 languages spoken (Urdu, English, Kashmiri, Balti, Shina, Burushahki, Chitrali, Kafri, Kohistani, Pushto, and Punjabi).

Methods

Information was gathered through field surveys, interviews, and secondary data. Primary data were collected in the field by the staff of the Himalayan Wildlife Foundation (HWF) in AJK and parts of NAs and NWFP (Table 1). During these surveys,

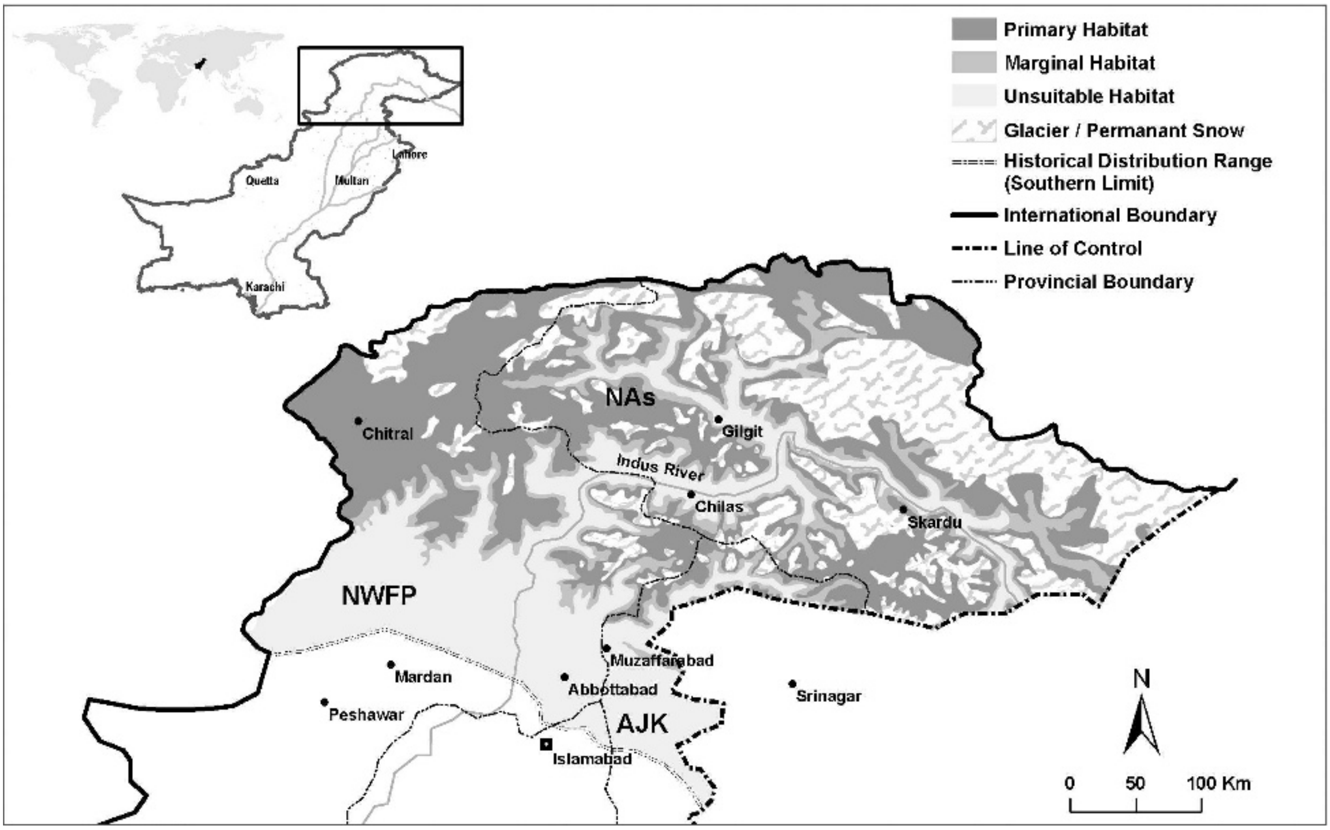

Fig. 1. Potential habitat of the brown bear in Pakistan, 2006.

line transects were placed to record sightings and signs of brown bears, and local people were interviewed. Line transects were usually 10–15 km long, and type of sign included scats, footprints, hair, digging, marks on trees, and damage to crops. The HWF gathered particularly good information from areas in the vicinity of Deosai, such as Gultari, Astore Valley, and also from the slopes of Nanga Parbat Peak and the Kaghan Valley. I also obtained data from the staff of the Deosai brown bear project who collected data relevant to the presence of bears as they worked in the region between 1994 and 2005.

I did not use a structured questionnaire for the interviews; rather, I targeted people in local communities, mountain nomads (*gujjars*), field staff of wildlife or forest departments, tourist operators (particularly for glacier areas), wildlife biologists, and relevant institutions and organizations. The field teams helped in collecting information from local communities and nomads, whereas the office-based relevant personnel were interviewed by me. I consulted personnel from Northern Areas Forestry, Parks and Wildlife Department (NAFWD), NWFP Wildlife Department (NWFPWD), AJK Department of Fisheries and Wildlife (AJKWD), National Council for the Conservation of Wildlife (NCCW), Pakistan Museum of Natural History (PMNH), Zoological Survey Department (ZSD), military on the India–Pakistan border, the Himalayan Jungle Foundation, The World Conservation Union (IUCN), and World Wide Fund for Nature Pakistan. I obtained additional secondary data from published and unpublished literature. I used *Survey of Pakistan* topographical maps (Survey of Pakistan 1997) to estimate potential brown bear habitat in Pakistan. The historical distribution range is based on Erdbrink (1953) and Servheen (1990), which I adjusted using the topographical maps and reported evidence.

Results

Historic range

U. a. isabellinus historically occupied the western Himalaya, the Karakoram, the Hindu Kush, the Pamir, the western Kunlun Shan, and the Tian Shan

Table 1. Field surveys conducted by the teams from Himalayan Wildlife Foundation for determining status of the brown bear, 1993–2006.

Details of survey	Date	Length (days)	Methods	Areas covered	Documentation
Annual census of the brown bear population in Deosai National Park, Northern Areas	1993–2006	15–20 days each year	Line transects, observations of individuals	Deosai National Park	Counted 43 individuals in 2006
Large mammal surveys in the Neelam Valley, AJK	Aug–Sep 2005	2005: 9 days	26 line transects	Dudgai, Gurez, Halmat, Pulwai, Sardari, Shontar, Surgun, and Gumot	2005: 5 bears sighted, 2 bears reported illegaly shot
	Aug–Sep 2006	2006: 21 days	35 Interviews		2006: 17 signs recorded for black and brown bears, 5 confirmed for brown bears
Wildlife surveys in the Minimerg Valley, Northern Areas	Sep 2005 Oct 2006	2006: 8 days 2005: 15 days	11 line transects 6 interviews	Dudgai, Minimerg, Shaban Top	10 sightings, 14 signs recorded
Wildlife survey in the Siran Valley, NWFP	23–30 Sep 2006	8 days	4 transects 15 interviews	Siran Valley, District Mansehra	No evidence for brown bears. Local people reported extirpation from the area.
Reconnaissance survey of the Neelam Valley, AJK	Nov 2005	8 days	3 line transects 12 interviews	Gumot National Park, Kel, Shontar and Gurez valleys	3 signs recorded; local people reported sightings
Survey of the Central Karakoram National Park (HBP, unpublished report, 2005), Northern Areas	15 Sep–15 Oct 2005	30 days	2 transects 61 interviews	13 valleys: Hushe, Thalley, Shigar, Askole, Arandu, Tormik, Stak, Haramosh, Gilmit, Minapin, Hoper, Hispar, and Shimshal	Bear presence reported in Shigar, Braldu (Ho Nala), and Baltoro glacier areas
Survey of Biafo and Panmah glaciers in Karakoram Range, Northern Areas	Aug 1998	12 days	Long tracking	Biafo and Panmah glaciers	Sighting of 1 bear, and 2 signs
Survey of Bar Valley, Gilgit	Jun 1997	10 days	Long tracking	Bar Valley	Signs of 1 female with cub, and 1 large bear were recorded

ranges in southern Asia. In Pakistan the subspecies ranged over the approximately 150,000 km^2 northern part of the country. They have been reported in several localities in the western Himalaya, including the Neelam Valley north of Machiara National Park, the Kaghan Valley, the Astore Valley, Nanga Parbat, and the Deosai Mountains. Their presence was also recorded in peripheral valleys, high meadows, and glaciers in the Karakoram, Hindu Kush, and Pamir ranges (Schaller 1977, Rasool 1982, Wegge 1988, Roberts 1997), as well as on the inter-mountain highlands of Indus Kohistan, Swat Kohistan and probably Hindu-Raj mountains (Servheen 1990, Roberts 1997). Bears also occurred in the south as far as the Hazara (Roberts 1997) and Waziristan areas (Ellerman and Morrison-Scott 1951), but seem to be extinct there now.

Potential habitat

In the Himalaya, brown bears inhabit mainly subalpine and alpine areas between 2,600 and 5,000 m (Schaller 1977, Roberts 1997, Sathyakumar 1999), where blue pine (*Pinus wallichiana*) forests (spring and fall) and alpine meadows (summer) are their primary habitats. Areas above these elevations are usually permanently covered with snow and are not suitable bear habitat. Alpine meadows are limited in the southern part of the range of brown bears in Pakistan, but forests become more prevalent, for instance in the Neelam and Kaghan valleys, where brown bears are sympatric with Asiatic black bears. Dominant tree species are blue pine, spruce (*Picea smithiana*), silver fir (*Abies pindrow*), and deodar (*Cedrus deodara*). Broadleaved trees that are intermixed with conifers, particularly in the riparian zones, include *Aesculus indica*, *Ulmus wallichiana, Juglans regia, Quercus floribunda, Acer caesium*, and *Prunus cornuta*. In Pakistan, the area where alpine meadows are prevalent (between 3,000 and 4,600 m) covers about 51,000 km^2, whereas the blue pine zone (2,600–3,000 m) covers about 19,000 km^2. Therefore, I infer that the potential habitat for brown bears in Pakistan is approximately 70,000 km^2 (Fig. 1). This may be an overestimate, as the western part of the range is dry and forest cover there is quite low.

Present population status

Brown bears have been extirpated from the majority of their historical range in Pakistan and currently exist only in small pockets. Today approximately 150–200 bears may survive in Pakistan in 7 populations. Connectivity among these populations is limited and some are completely isolated. Populations and subpopulations have been defined following Zedrosser et al. (2001). The present status of the Pakistani brown bear populations is summarized in Table 2 and Fig. 2.

Northern Areas. Three populations and 5 subpopulations can be identified in NAs (Fig. 2, Table 2). The Himalayan population is the largest, followed by the Karakoram population, whereas the Hindu Kush population is very small.

The western Himalaya in NAs hosts 3 subpopulations, referred to as the DNP, Minimerg, and Nanaga Parbat. The DNP is the largest subpopulation, consisting of about 40 individuals. This subpopulation occupies the main Deosai Plateau and surrounding 6 valleys: Karabosh, Dhappa, Shilla, Shagarthang, Bubind, and Chillam. The Minimerg subpopulation exists east of the Deosai along the line of control (LOC). It covers the localities of Burzil Pass, Shaban Top, Gultari, Minimerg, and Kamri. This area is characterized by narrow valleys, steep slopes, and some good forest stands. A bear was shot on Shaban Top in 2000, the HWF staff recorded bear sign frequently in the Gultari area during the last 6 years, and a bear was sighted in early spring 2003. I observed a female with a cub in the Minimerg Valley during the September 2005 survey, and HWF staff frequently encountered bear sign in the Dudgai and Kamri areas. Local villagers reported many bears in the area, and an officer of the Pakistan Army reported a bear crossing the LOC between Indian Kashmir and NAs of Pakistan in 2004. Approximately 10–15 individuals occupy this area. The third subpopulation of Himalaya is present around the slopes of the Nanga Parbat Peak, including localities such as Babusar Pass, Raikot Valley (Fairy Meadows), Astore Valley, and Rattu, Kalapnai. I estimate about 10 bears in this area.

Two subpopulations of brown bears are found in the Karakoram Range: one in the Central Karakoram National Park (CKNP) and the second in the Khunjerab National Park (KNP). In CKNP brown bears are reported in low densities from Shigar, Baraldu (Ho Nala), and Baltoro Glacier (Hagler Bailly Pakistan, 2005, Central Karakoram Protected Area: Volume II baseline studies, Draft Report Prepared for IUCN Pakistan, Karachi, Pakistan) and also from Nagir, Chaprote, Bar Nallah (Rasool 1982, 1991). Observation of one bear and some sign

Table 2. Distribution of brown bear in Pakistan, approximate population size and trend, 2006.

	Province	Population	Sub-population	Localities	Approximate size	Status
1a	Northern Areas	Himalayan	Deosai National Park (DNP)	DNP and surrounding valleys; Karabosh, Shilla, Dhappa, Sadpara, Shagarthang, Bubind, and Chilam	40–50	Stable
1b			Minimerg	Minimerg, Burzil, Kamri, Shaban Top	10–15	Declining
1c			Nanga Parbat	Astore and Raikot valleys, Rattu, Kalapani	10	Declining
2a		Karakoram	Central Karakoram National Park	Shigar (Braldo, Basha), Glaciers (Baltoro, Biafo, Panmah), Nagir, Chaprote, Bar Nallah, Kilik, Minteka	25	Declining
2b			Khunjerab National Park	Barakhun nullah, Khunjerab Pass, Sherlik area near Oprang River	10–15	Declining
3a		Hindu Kush	Ghizer	Ghizer, Singal, Chassi	10	Declining
3b			Karambar	Karambar Lake, Karambar River (behind the Chiantar Glacier, close to border with Afghanistan)	5–10	Declining
3c	North West Frontier Province		Tirch Mir	Upper part of Yarkhan River, and along the border with Afghnistan	5–10	Declining
3d			Chitral	Chitral Gol National Park	Extinct	Extinct
4		Kalam			~5	Declining
5		Indus Kohistan		Palas Valley and adjacent areas	~5	Declining
6		Kaghan		Kaghan Valley including Dodopat National Park	8–10	Declining
7		Hazara		Siran Nalla	Extinct	Extinct
8	Azad Jammu and Kashmir	Machhiara National Park			Extinct	Extinct
9a		Neelam Valley	Gumot	Gumot National Park, Surgun Valley	5–10	Declining
9b			Shontar Valley		~5	Declining
9c			Gurez Valley	Taobat, Halmat, Gugai	10–15	Declining

were recorded from Biafo and Panmah glaciers (Himalayan Wildlife Foundation 1999, W.L. Gaines, US Forest Service, Wenatchee, Washington, USA, personal communication, 2005), and also some sign from the Bar Valley during a survey in 1997. A population of 25 bears may roam in the vast area of CKNP. In KNP, bears have been reported from Barakhun Nullah, Khunjerab Pass, Sherlik area near Oprang River, Kilik, and Minteka (Schaller 1977, Wegge 1988, Ahmed 1989, Rasool 1991). One bear was observed in Khunjerab Nullah (Z.B. Mirza, Centre of Environment Research and Conservation, Islamabad, Pakistan, personal communication, 2005), and recently a brown bear was photographed with a remote camera set to record snow leopards. The population in KNP is probably 10–15 individuals.

The third population exists in the Hindu Kush Range, with 3 declining and 1 extinct subpopulations. Schaller (1977) collected 6 bear scats from the Karambar Lake, located at the source of the Karambar River, behind the Chiantar Glacier, close to the border with Afghanistan (Wakhan Corridor). In the Gizer area, bears may exist in the main Gizer Valley, and also in Singal and Chassi (Rasool 1991). Each of the Ghizar and Karambar subpopulations probably consists of 8–10 bears.

Azad Jammu and Kashmir. Brown bears in Northern Kashmir are restricted to the Neelam Valley, in the recently created District Athmakam (old District Muzaffarabad). Alpine and sub-alpine pastures are 2 major categories of the land use in this area, where the habitat is under heavy grazing pressure and over time the productivity and biodiversity has declined. Brown bears are unlikely to inhabit areas south of Gumot National Park because there is no suitable habitat available. Presently they occupy only the northern part of this valley including the Gumot, Shontar, and Gurez valleys, and the Kel Area. The Gurez Valley particularly has excellent habitat conditions and bear signs were encountered more frequently in this area. Relatively intact forest (with dominant species as *Pinus wallichiana, Picea smithiana, Abies pindrow*, and *Cedrus deodara*) along the left bank of the Neelam (Kishangana) River is of high importance for brown bears, particularly in the Hanthi, Halmat, and Gugai areas. This area is along the LOC between India and Pakistan. An HWF team observed 3 bears in the Surgun Valley (including a female with a cub) and 2

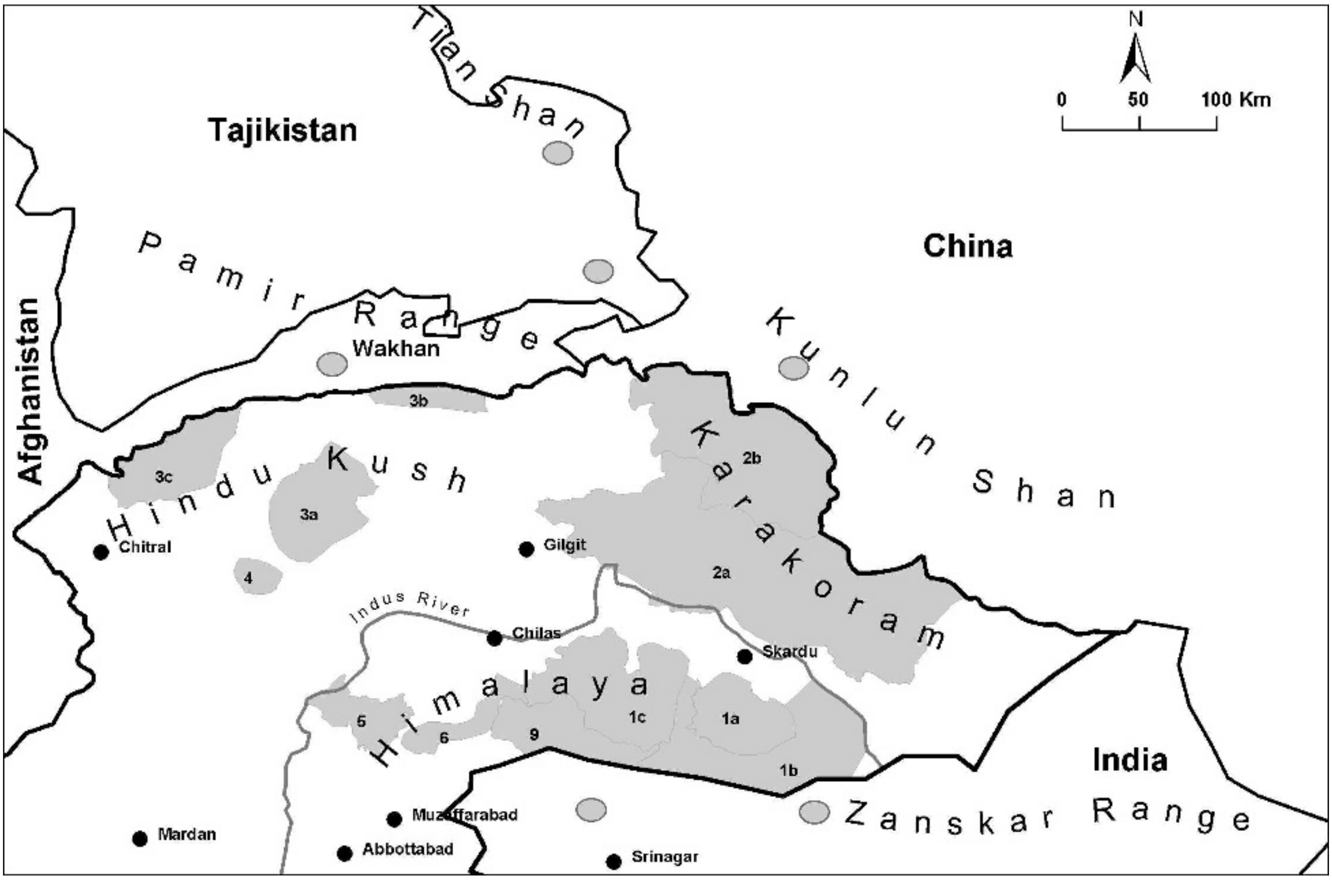

Fig. 2. Distribution of brown bear populations in Pakistan, 2006. Grey circles represent populations reported outside Pakistan. Numbers refer to brown bear populations and sub-populations from Table 2.

bears in the Gurez Valley, and spoor was collected from the northern part of the Neelam Valley during 2004–2006. Local people and nomads (*gujjaras*) also report frequent sightings of brown bears in this area. Two brown bears were illegally shot in Gurez Valley in August 2005 by a local hunter. A dead brown bear was found buried in debris; this bear probably died during the 2005 earthquake. The brown bear population is estimated at 20–25 individuals in this valley.

NWFP Province. The North West Frontier Province (NWFP) spans slightly over 100,000 km^2, with elevations ranging from 250 m to >3000 m (GoNWFP and IUCN 1996). Brown bears are restricted to northern NWFP, adjacent to the NAs populations. Brown bears occupy the Hindu Kush Range in the northern part of the Chitral District, the Kalam area in Swat Kohistan, Kaghan Valley, and Pallas Valley in Indus Kohistan (Arshad 2003). There are 3 populations (Kalam, Indus Kohistan, and Kaghan) and 2 subpopulations (Tirch Mir, Chitral) of the Hindu Kush population in NWFP. A population reported from Siran Nalla in Hazar District, and the subpopulation in Chitral Gol National Park are extinct (Schaller 1977, Mirza 2003). A small subpopulation of Tirch Mir still persists in the headwaters of Yarkhun and along the Afghan border. Fulton (1903) reported that brown bears were common in Turkho and Yarkhun valleys, and also Schaller (1977) observed some signs in this area. Local staff of the IUCN's Mountain Areas Conservancy Project (MACP) project also believes some bears are surviving in this area.

Regional connectivity. Brown bears survive in all neighboring countries; however, their range is no longer contiguous. Populations in the entire region are largely fragmented, but some populations may have some gene flow. Pakistani populations, which occupy the southern limit of the brown bear distribution, seem to have limited contact with neighboring populations toward the north and east.

Toward the east, brown bears exist in India and perhaps in Nepal (Gurung 2004). In India, they are confined to the northwestern Himalaya in Jammu, Kashmir, Himachal Pradesh, Uttar Pradesh, and Sikkim, but there is poor information on population status from most of the range (Sathyakumar 1999, 2001; Johnsingh 2003; Kaul et al. 2004). Points of contact between the Indian and Pakistani populations are the Zanskar and Ladakh ranges and the Gurez Valley (northern part of the Neelam Valley). Exchange through the Karakoram Range is unlikely, because brown bears do not exist on the Indian side of this range (S. Sathyakumar, Wildlife Institute of India, Dehradun, India, personal communication, 2005). Our recent observations in the Neelam and Minimerg valleys reveal that animals cross the Indian–Pakistan border. Military presence and tension on the LOC have been beneficial in a way, because it restrained the expansion of human population and related infrastructure and halted natural resource depletion in these areas since partition in 1947.

Toward the north and northwest, brown bears occupy the Kunlun and Tian Shan ranges. A number of studies have documented presence of brown bears in the Tian Shan Range, including parts of Tajikistan, Uzbekistan, Kyrgyzstan, Kazakhstan, and China (Ministry of Environmental Protection 1998, Glukhovtsev and Yermekbayeva 2001, P. Wegge, Norwegian University of Life Sciences, Ås, Norway, personal communication, 2006), where it is sometimes referred to as the Tian Shan brown bear (Dexel 2002). Vaisfeld and Chestin (1993) estimated 2,000–3,000 bears in the Central Asian states, and described 3 subspecies. In Tajikistan, an estimated 700 brown bears occur in the Pamir and Alai mountains (Vaisfeld and Chestin 1993). Brown bear signs were observed in a recent survey in the Wakhan Corridor in northeastern Afghanistan (Mishra and Fitzherbert 2004). The bear population in the Wakhan Corridor is a crucial link between the Hindu Kush population in Pakistan and the Central Asian populations. Brown bears also survive in Kunlun Shan in China (Schaller 1998, Harris and Loggers 2004). Brown bear movement is likely to occur between the Karakoram and Kunlun ranges, as they are adjacent and both are occupied by bears.

Considering the geomorphology of the area and the reported evidence, I conclude that the Pakistani populations of brown bears exhibit regional connectivity primarily through 3 corridors: the Himalayan population is connected to the populations in Zanskar and Ladakh ranges in India, the Karakoram population has connectivity with Kunlun Shan in China, and the Hindu Kush population is connected to bears in the Tian Shan Range through the Pamir population in the Wakhan Corridor (Afghanistan) and Central Asia (Fig. 2).

Discussion

Brown bears in Pakistan are declining because of habitat loss and fragmentation, human-induced mortality, commercial poaching for the sale of bear parts, bear baiting, and poaching of bear cubs for sale to gypsies.

Habitat threats

Pakistan became the world's ninth most populous country in 1994, and, at 2.1% per year in 1998, has one of the world's highest population growth rates (Population Census Organization 2001). The population has reached 142.5 million, from 16.6 million in 1901, and is projected to double by 2035 (Faizunnisa and Ikram 2002). This human pressure is obvious even in NAs, where population growth rate has been estimated at 2.47% per year (GoP and IUCN 2003) and where the population has quadrupled since the creation of the state in 1947 (Ehlers and Kreutzmann 2000). The environmental consequences of rapid population growth are pervasive, and the increases in demands for natural resources and their subsequent depletion have many consequences for bears and other wildlife. The increase in the size and number of settlements, expansion and improvement in infrastructure, transformation of land use, and attenuation of forest cover are the major factors which contributed to the significant shrinking and fragmentation of the bear habitat during the last 5 decades. Forests are being cut for timber and firewood and cleared for increasing areas for cultivation. Bear utilize alpine meadows more than any other vegetational zone in NAs, where they constitute around half of the available land. However, in NAs such meadows have experienced accelerated transformation in the last 2 decades (Kreutzmann 1991, 1995). The natural grazing areas were estimated at 3.6 million ha in 1950, and were considered largely sufficient for a livestock population of 1.12 million animal units (Ehlers and Kreutzmann 2000). With livestock estimated at over 2 million in 1998, a shift in the availability of high altitude pastures has been observed, from abundant to 30% deficient (Ehlers and Kreutzmann 2000).

This has resulted in an obvious numeric and spatial expansion in nomadic and transhumance grazing in alpine pastures.

Threats to bears

Hunting has been a traditional practice in most of the bear range in Pakistan. Increasing accessibility and number of vehicles has increased the hunting of wildlife. As a consequence, bears and other large mammals have been largely eliminated in the areas near settlements. Despite the ongoing protection efforts in areas like Deosai National Park, human-induced mortality continues and a minimum of 9 bears were killed in the 10-year period 1996–2005, (3 males, 4 females, and 2 cubs). Bears have been hunted for sport (usually by military officers), persecuted by villagers who feel their livestock is threatened, and more recently killed for commercial purposes. At least 5 sites were identified in Gilgit, Sakardu, and other towns along the Karakoram Highway (HWF 1999) where bear fat was sold on a regular basis for about 60 Pakistan Rupees (PKR) per *tola* (16 grams) (US$ 62.5/kg; 2006 rate). It is estimated that bear parts from an adult bear could fetch as much as PKR 75,000 (US$ 1,250; 2006 rate) in a local market (Himalayan Wildlife Foundation 1999), which is much higher than the annual income of a typical wage earner in the NAs. This provides a strong incentive for bear poaching. Female bears are also killed to capture their cubs for sale to gypsies. Cubs of the year are preferred, as they are easy to train for bear displays and baiting events. Nomad graziers (*gujjars*), who travel all the way from the plains to the mountains with their livestock, are known to be involved in this business in addition to other illegal activities, like collection of medicinal plants. Graziers are suspected to transport poached wildlife down to the plains.

Threats of changing climate

Brown bears are potentially threatened by impacts of climate change. Potential threats include loss of habitat, decline in food supply, habitat shift to non-protected areas, and increased competition with humans. The major habitat of brown bears in Pakistan is the alpine cold desert zone that lies in the alpine tundra biome. The computer simulation model BIOME3 predicted changes in the size and location of forest ecosystems and biomes of Pakistan under the influences of climate changes (increase in temperature and rainfall scenarios) in the year 2020 and 2040–50 (Hagler Bailly Pakistan 1999). In general, the model predicted a positive effect on the forests of Pakistan, but alpine tundra, which covers about 6.8% of the total area, would be reduced to 4.6% by the year 2020. A northward and upward shift of all biomes is predicted. The coniferous biome is expected to expand at the expense of alpine tundra. Brown bears already suffering habitat degradation and fragmentation by anthropogenic activities will face further shrinkage of habitat, and this could have serious consequences on their survival.

Management framework

Pakistan has ratified the Convention on Biological Diversity (CBD), and as a follow up, developed the National Conservation Strategy (NCS) and Biodiversity Action Plan (BAP) for environmental protection and biodiversity conservation. Wildlife conservation is the responsibility of the provinces in Pakistan, and each province has its own legislation, which is implemented by its respective wildlife or forest department. The brown bear range in northern Pakistan is managed by 3 provincial departments: the NAs Forestry, Parks and Wildlife Department; the NWFP Wildlife Department; and the AJK Department of Fisheries and Wildlife. The National Council for Conservation of Wildlife (NCCW) in the Federal Ministry of Environment, Local Government and Rural Development is responsible at the national level for the coordination of the provincial conservation programs in order for Pakistan to fulfill its international obligations and agreements regarding biodiversity conservation.

Three wildlife laws are effective in northern Pakistan: the Azad Jammu and Kashmir Wildlife Act (1975), the Northern Area Wildlife Preservation Act (1975), and the NWFP Wildlife (Protection, Preservation, Conservation and Management) Act (1974). These acts provide the basis for the creation of protected areas in 3 fundamental categories: national parks, wildlife sanctuaries, and game reserves. All provinces have made considerable process in the establishment of protected areas (PAs) that provide legal cover for the protection and conservation of a variety of wildlife; 7 national parks, 8 wildlife sanctuaries, and 10 game reserves have been established in brown bear range in Pakistan (Fig. 3). These PAs cover the majority of the existing brown bear populations and provide them with legal protection against hunting and other

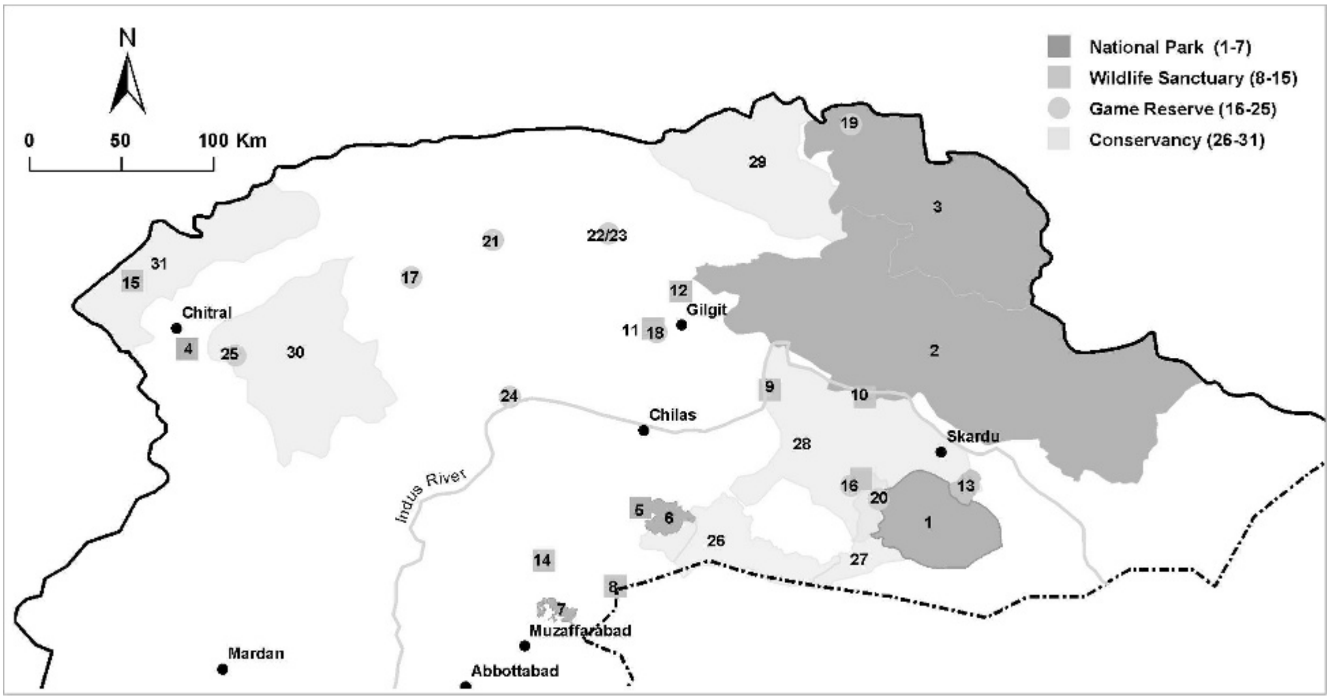

Fig. 3. Network of protected areas in Northern Pakistan, 2006.

threats. However, except for a few of those areas including the DNP and the KNP, which are effectively managed, these PAs unfortunately just exist on paper. They were created haphazardly and face problems like weak law enforcement, poor institutions and infrastructure, and lack of adequate resources. Among a total of 25 PAs in northern Pakistan, 16 lack basic baseline information, 22 do not have any management plan, and 19 are without any management infrastructure.

Conservation recommendations

The bear population in Pakistan has shrunk radically and continues to decline in its entire range, with only the exception of Deosai National Park. Immediate efforts are needed to ensure its long-term survival, which will be more effective if taken jointly by the state departments, non-governmental organizations (NGOs), research institutes, and communities.

Because most existing bear populations are covered either by the PAs or conservancies, there is no need to create additional protected areas, at least in the short term. However, with limited financial resources and ineffective protection and management systems, these PAs carry little meaning. The World Conservation Union (World Conservation Union 2000) reviewed PAs of Pakistan, and through a process of wide consultation (Ghazali and Khairi 1994) developed a comprehensive action plan framework for strengthening the PAs system and improving its efficiency. The framework identifies priorities for actions and investment, sets definable and measurable goals, and can be smoothly integrated into long-term national policy. The only thing lacking is its implementation and adoption by the concerned departments and authorities.

Carnivores as a whole are considered odious and it is usually difficult to generate support by local communities for their conservation. People always question such efforts because, unlike ungulates, carnivores don't have any meat value and pose a threat to humans and livestock. Environmental education is an important instrument to change perceptions and attitudes. Launching education and awareness initiatives that cater to local communities, staff of the PAs, visitors, and the general public can bridge the knowledge gap and be vital to achieving synergy in conservation efforts. Trophy hunting in Pakistan is an increasingly popular tool for conservation through community participation. Presently based on 5 ungulate species, this program has generated substantial revenue which has been shared with local communities. The trophy hunting program has been effective in rehabilitating populations of wild ungulates; however, its contribution to the conservation of biodiversity as a whole is limited. The programs' impact on bears is perhaps neutral, while other predators like snow leopards and wolves have been negatively affected (Hussain 2003). This program can play a significant role if conservation of carnivores is integrated in the approach. For example, linking trophy hunting quotas, which are fixed by the federal government annually, to the populations of threatened carnivores in addition to the population of trophy animal, would be an effective step.

Human population growth, infrastructure development, forest depletion, and many other related factors have consequences for the bear population. The growth in number of livestock and increasing dependency on alpine pastures is the major threat to bears, and increasingly generates human–bear conflicts. Appropriate management of this issue will largely determine the future of this species in many areas.

Management of the Himalayan brown bear on an international scale is central to ensure its survival in the long run. The Neelam Valley and the Pamir Knot are 2 ideal venues for cross-border cooperation for conservation. The Neelam Valley has been designated as a conservancy and a proposal is being worked out to create 2 new protected areas in its northern segment (Gugai and Gurez National Parks). Protection on the other side of the LOC in India would help conservation across the natural range and uphold the possibility of bear movements in the future. A peace park around the Pamir Knot (the area in northern Pakistan where all mountain ranges come together), involving Afghanistan, Tajikistan, China, and Pakistan, is also under consideration (U. Khalid, NCCW, Islamabad, Pakistan, personal communication, 2005). Dr. G. Schaller has been instrumental for this initiative, and the conservation of Marco Polo sheep (*Ovis ammon polii*) is its primary target. If this proposal is successful, this park will not only potentially allow for an increase in the bear population, but also safeguard the corridors with the Kunlun and Tian Shan ranges.

Deosai National Park should remain the focus of conservation efforts, because the future of the brown bear in the country will largely depend on stability in this park. The role of the Deosai population is somewhat analogous to a mainland or source population in a metapopulation context. It is important to work simultaneously on improving habitat quality in Deosai and on improving its connectivity with neighboring populations. Better connectivity will protect populations from inbreeding depression and will increase the colonization rate in the Himalaya. Suitable corridors in the range should be identified and maintained to facilitate dispersal.

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Genetic tracking of the brown bear in northern Pakistan and implications for conservation

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Asian bears face major threats due to the impact of human activities as well as a critical lack of knowledge about their status, distribution and needs for survival. Once abundant in northern Pakistan, the Himalayan brown bear (*Ursus arctos isabellinus*) has been exterminated in most of its former distribution range. It presently occurs sparsely, in small populations, the Deosai National Park supporting the largest isolate. This decline might imply a reduction in genetic diversity, compromising the survival of the population. Using a combination of fecal DNA analysis and field data, our study aimed at assessing the size and genetic status of the Deosai population and give guidelines for its conservation and management. Using fecal genetic analysis, we estimated the population to be 40–50 bears, which compares well with the field census of 38 bears. The northern Pakistani brown bear population may have undergone an approximate 200–300-fold decrease during the last thousand years, probably due to glaciations and the influence of growing human population. However, in spite of the presence of a bottleneck genetic signature, the Deosai population has a moderate level of genetic diversity and is not at immediate risk of inbreeding depression. Gene flow might exist with adjacent populations. We recommend careful monitoring of this population in the future both with field observations and genetic analyses, including sampling of adjacent populations to assess incoming gene flow. The connectivity with adjacent populations in Pakistan and India will be of prime importance for the long-term survival of Deosai bears.

1. Introduction

Brown bears (*Ursus arctos*) are the most endangered and least studied in Asia, where populations have declined by more than half in the past century (Servheen, 1990; Servheen et al., 1999). Asian bears face threats due to the impact of human activities and there is a critical lack of knowledge concerning their status, distribution and requirements for

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survival (Servheen et al., 1999). The Himalayan brown bear (*U.a.isabellinus*), a highly threatened subspecies, is distributed in small isolated populations over the Himalaya, Karakoram, Hindu Kush, Pamir, western Kun Lun Shan, and Tian Shan ranges in southern Asia.

This bear has been exterminated in most of its former distribution range in Pakistan, and occurs very sparsely in small populations with limited connectivity in northern mountainous areas. Deosai National Park is the main stronghold of the brown bear population in Pakistan (Schaller, 1977; Roberts, 1997). Once abundant in Deosai, bear numbers declined drastically to as low as 19 in 1993 (Himalayan Wildlife Project, 1994). Although the population in Deosai has been recovering gradually since 1993 due to strict protection and conservation efforts, the decline could have reduced the genetic variability considerably. As a consequence, this population might suffer from inbreeding, and its survival might be compromised. Small population size is a great concern in conservation biology because small populations are more vulnerable to genetic factors, demographic and environmental stochasticity, genetic drift and inbreeding and have increased probability of extinction (Soulé, 1987). Evolutionary processes such as mutations, migration, selection and stochasticity are also fundamentally different than those in large populations. In small populations the role of stochasticity increases and the impact of selection is limited (Frankham et al., 2002). The loss of genetic diversity as a result of a bottleneck or continued small populations has been documented in many endangered species such as the northern elephant seal (*Mirounga angustirostris*) (Bonnell and Selander, 1974), Mauritius kestrel (*Falco punctatus*) (Groombridge et al., 2000), Indian rhinoceros (*Rhinoceros unicornis*) and Siberian tiger (*Panthera tigris*) (Hedrick, 1992). Fragmented populations are prone to many subtle threats, such as limited dispersal and colonization and restricted access to food and mates (Primack, 2002).

Documenting the status and distribution of Asian bears has been identified as a priority action for conservation by the IUCN/SSC Bear Specialist Group (Servheen et al., 1999). A comprehensive action plan is required for the long-term management of Himalayan brown bears. In order to be effective, an action plan should be based on reliable biological data, such as trustworthy estimates of population size, population genetic status and connectivity with other populations. Population size estimates are difficult to obtain for rare and elusive animals like brown bears (Bellemain et al., 2005). Field methods based on observations of recognizable individual bears have been used to estimate the size of the Deosai population, but these methods have not been compared with censuses using independent methods in order to evaluate their consistency.

To assess the genetic status and size of the Deosai population and give guidelines for the conservation and management of this population, we used the increasingly popular non-invasive genetic technique (Taberlet et al., 1996, 1999), in combination with field data. Using DNA analyses of fecal sampling, we aimed to answer the following questions: (i) Is the population size estimated from field data consistent with genetic censuses? (ii) Did the population suffer from a bottleneck at the genetic level and how long ago did it begin to decline? (iii) Are Deosai bears at risk of inbreeding depression? (iv) Is the population genetically isolated?

2. Material and methods

2.1. Study area and studied populations

The study was conducted in the Deosai National Park, Northern Areas, Pakistan. Deosai National Park is a plateau in the alpine ecological zone encompassing about 20,000 km^2, situated 30 km south of Skardu and 80 km east of the Nanga Parbat Peak. Elevations range from 3500 to 5200 m and about 60% of the area lies between 4000 and 4500 m. Recorded mean daily temperatures range from −20 °C to 12 °C. The annual precipitation in Deosai is 510–750 mm, and falls mostly as snow (Himalayan Wildlife Foundation, 1999a). The Deosai plains are covered by snow during winter months between November and May, and life on the plateau is confined to a window of five months.

The Deosai Plateau is situated between two of the world's major mountain ranges, the Karakoram and Himalaya. The biota includes plants and animals from Karakoram, Himalaya and Indus Valley. As a result Deosai is a center of unique biota in northern Pakistan. The documented biota of Deosai National Park includes 342 species of plants, 18 of mammals, 208 of birds, three of fishes, one of amphibian, and two of reptiles (Woods et al., 1997). Most of the plant species are herbaceous perennials, and cushions forming and tufted plants are common growth forms. Plains present a mosaic of plant communities according to the availability of water. The low lying areas usually consist of bogs and pools with associated flora consisting predominantly of grasses and sedges and plants such as *Saxifraga hircus*, *Swetia perfoliata* and *Aconitum violaceum*.

Deosai National Park supports the largest population of brown bears in Pakistan (unpublished data). The brown bear population in this park has been protected and closely monitored since 1993, and primary data on population size, behavior and ecology have been gathered (Himalayan Wildlife Foundation, 1999b). Field personnel were able to recognize dominant bears from their physical characteristics, coloration and well defined home ranges on this open plateau (Himalayan Wildlife Foundation, 1999a,b; Nawaz et al., 2006). Based on this, they estimated the number of bears annually, the approximate age of some males and females, as well as their reproductive behavior and, in some cases, relatedness (mothers and their young).

2.2. Fecal sampling

The study area was searched for bear feces from July to early October 2004. We divided the study area into five blocks, and each block was searched for bear feces in order to cover most of Deosai National Park (Fig. 1). Transects of 40–60 km length were placed in each block, and walked by a team of 2–3 people. The transect routes were planned in a way that these covered the maximum extent of the block and passed through areas known for frequent bear sightings. Transect routes usually resembled a loop, starting from the central road, progressing towards periphery of the park, and ended at the starting point. The team walked along opposite borders of a block while going towards the periphery of the park and coming back to the road. Each transect was completed in 2–3 days,

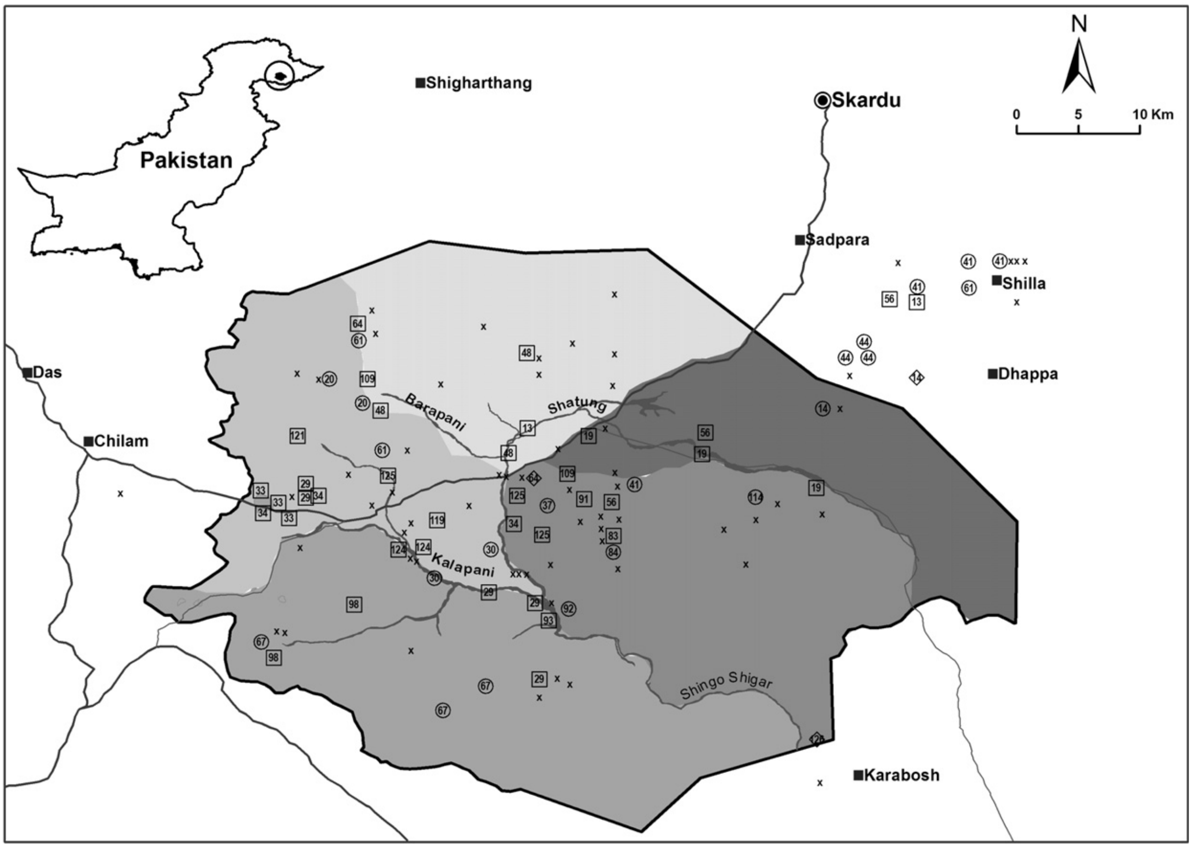

Fig. 1 – Map of the study area in the Deosai National Park, Northern Areas, Pakistan. Spatial distribution of brown bear genotypes is represented with squares for males, circles for females, and diamonds for unknown sex. Numbers within squares or circles represent individuals' identification numbers. Samples with negative/poor amplification are shown as "x". Five survey blocks are represented by different shades of grey.

with night stays made in portable tents. Apart from this planned collection, the field staff of Deosai National Park collected samples during their normal patrolling of the park.

Brown bears exhibit altitudinal migration in Deosai, and spend part of their life in surrounding valleys. We therefore collected feces from valleys connected to the park. When we found many feces together, usually at a bedding site, we collected one sample from the freshest feces. However if several feces were found at a food source (e.g. carcass) or we could differentiate different sizes, we took multiple samples. We picked up each fecal sample with a stick of wood and put 1 $cm^3$of it in a 20-ml bottle. For each fecal sample, a sampling date, a geographical location and coordinates (latitude/longitude) were recorded using a GPS receiver (Garmin 12XL). Bottles were then filled with 95% alcohol to preserve the samples until DNA extraction.

Approximate ages of fecal samples were evaluated on the field and categorized into five classes; (1) fresh feces of the same day, (2) two–three days old, (3) one week old, (4) feces of the same month, and (5) feces older than one month.

2.3. *DNA extractions and typing*

2.3.1. *Extraction*

For every collected fecal sample, DNA extractions were performed using the Qiamp DNA Stool Kit (Qiagen, Hilden, Germany), developed especially for this type of material and following the manufacturer's instructions. All extractions occurred in a room dedicated to processing hairs and feces. Tubes containing samples and tubes without feces were treated identically to check for exogenous DNA contaminations.

2.3.2. *Genotyping for individual identification*

The extracted DNA was amplified using the six microsatellite primers described in Bellemain and Taberlet (2004) on a set of 16 feces to test for their polymorphism. The number of alleles per locus ranged from one to eight. The two primers showing only one or two alleles (Mu10 and G10L) were discarded for this analysis (but included later, see below) and the four others (Mu23, Mu50, Mu51, and Mu59) were kept. In order to obtain a probability of identity low enough to differentiate among all individuals, we redesigned two other microsatellite primer pairs, namely G10J and G10H (from Paetkau and Strobeck, 1994; Paetkau et al., 1995):

G10HFIpak: GGAGGAAGAAAGATGGAAAAC
G10HRpak: AAAAGGCCTAAGCTACATCG
G10JFpak: GCTTTTGTGTGTGTTTTTGC
G10JRIpak: GGTATAACCCCTCACACTCC

For sex identification, we used the SRY-primers described in Bellemain and Taberlet (2004).

We simultaneously amplified the following loci: Mu23 with Mu50; SRY with Mu51 and Mu59; G10Jpak with G10Hpak, using the internal fluorescent primers together with the appropriate external primers. We repeated each amplification eight times following the multi-tube approach (Taberlet et al., 1996). The fluorescent PCR products were loaded together on the single electrophoresis (ABI Prism 3100 DNA sequencer; Applied Biosystems, Foster City, California). The gels were analyzed using Genemapper (version 3.0) software package (Applied Biosystems, Foster City, California). We typed samples as heterozygous at one locus if both alleles appeared at least twice among the eight replicates and as homozygous if all the replicates showed identical homozygous profiles. If neither of those cases occurred, the alleles were treated as missing data.

We calculated a quality index for each sample following the rules defined in Miquel et al. (2006). To be conservative, we discarded the samples that had a quality index below 0.5.

2.3.3. *Genotyping for population genetics analyses*

To estimate population genetics parameters and relatedness, we increased the number of loci for each genetically identified individual. The highest quality sample per individual was selected, based on quality indices when the individual was represented by several samples. We amplified the following 12 additional microsatellites: G1A, G1D, G10B, G10C, G10L, G10P, G10X, G10O (Paetkau and Strobeck, 1994; Paetkau et al., 1995) and Mu05, Mu10, Mu15, Mu61 (Taberlet et al., 1997), using a modified protocol from Waits et al. (2000). The amplifications were performed using five combinations of loci: (1) G10B, G10C (2) G10X, G10P; (3) Mu61, Mu05; (4) G10O, G10L (5) G1D, Mu15; loci Mu10 and G1A were amplified separately. PCR reactions of 12.5 μL containing 2 μL template DNA, 0.1 mM each dNTP, 0.5 μM of each primer, 3 mM MgCl 2, 0.5 U AmpliTaq Gold Polymerase (Applied Biosystems) and 1 × Taq buffer (containing 100 mm Tris–HCl, pH 8.3, 500 mm KCl, according to the manufacturer's specifications; Applied Biosystems). Amplifications were performed in a GeneAmp PCR system 9700 (Applied Biosystems) with the following conditions: 10 min at 95 °C, 35 cycles composed of 30 s denaturing at 95 °C, 30 s annealing at 57 °C for combination 1, 45 °C for combination 2, 48 °C for combination 3, 52 °C for combination 4, 55 °C for combination 5, 52 °C for Mu10 and 55 °C for G1A, 1-min extension at 72 °C, and as a final extension step, 7 min at 72 °C. We repeated each amplification four times. The PCR products were mixed in three multiplexes (1st: 2 μL G1A, 3 μL G10B/G10C, 5 μL Mu61/Mu05; 2nd: 3 μL G1D/Mu15, 7 μL G10P/G10X; 3rd: 5 μL Mu10, 5 μL G10O/G10L). One μL of this multiplex was added to a 10 μL mix of formamide and ROX 350 (10:0.2), and then loaded on an automatic sequencer ABI3100 (Applied Biosystems, Foster City, California). The gels were analyzed using Genemapper (version 3.0) software package (Applied Biosystems, Foster City, California). The same rules as described above were applied for defining homozygous and heterozygous loci.

A new quality index Miquel et al. (2006) was calculated for each sample and locus. The loci G10P, Mu05 and Mu61 were discarded from the analysis because of their low quality indices (below 0.6). Finally, genotypes were obtained based on 15 loci.

2.3.4. *Calculating the probability of identity*

Using the software GIMLET version 1.3.1 (Valière, 2002), and both datasets (6 and 15 loci), we computed the probability of identity, i.e. the overall probability that two individuals drawn at random from a given population share identical genotypes at all typed loci (Paetkau and Strobeck, 1994). We also computed the probability of identity among siblings (Waits et al., 2001).

2.3.5. *Estimating current population size using rarefaction indices*

Following the method described in Kohn et al. (1999), we compared the 6-loci genotype of each sample with all those drawn previously and calculated the population size as the asymptote of the relationship between the cumulative number of unique genotypes and the number of samples typed. This curve is defined by the equation $y = (ax)/(b + x)$, where a is the asymptote, x the number of feces sampled, y the number of unique genotypes, and b the rate of decline in the value of slope. Eggert et al. (2003) derived another estimator with a similar equation; $y = a(1 - e^{bx})$. These are referred to as the Kohn and Eggert methods, respectively. We analyzed data with the software package GIMLET version 1.3.1 (Valière, 2002), with 1000 random iterations of the genotype sampling order. Rarefaction equations were run using R software (version 1.7.1; available at http://www.r-project.org). Confidence intervals were calculated using the iterative approach, which is usually employed for rarefaction curves. However, this gives an indication of only the sampling variance and not the estimator variance.

2.3.6. *Investigating the genetic signature of the bottleneck*

At selectively neutral loci, populations that have experienced a recent reduction of their effective population size exhibit a characteristic mode-shift distortion in the distribution of allele frequencies (alleles at low frequency (<0.1) becoming less abundant; Luikart et al., 1998) and develop heterozygosity excess (Cornuet and Luikart, 1996). We used a Bayesian approach to detect and date a potential bottleneck in the Deosai bear population. This method is implemented in the MSVAR program (Beaumont, 1999) available at http://www.rubic.rdg.ac.uk/~mab. MSVAR calculates the Bayesian posterior distribution of demographic and mutational parameters, using a Markov Chain Monte Carlo approach. Mutations are assumed to occur under a stepwise mutation model with a rate $\theta = 2N_0\mu$, where μ is the locus mutation rate; the change in population size is assumed linear or exponential. The model assumes demographic history in a single stable population that was of size N_1 t_a generations ago and subsequently changed gradually in size to N_0 over the period from t to the current time. The program estimates two demographic parameters $t_f = t_a/N_0$ and $r = N_0/N_1$, where r indicates the population trend (population expansion if $r > 1$; population decline if $r < 1$).

For calculations we used the exponential growth models with the default parameters, as it is more suitable than the linear growth model for modeling population changes over a shorter time scale (Beaumont, 1999). For each population, 2×10^8 updates were calculated and only the last 90% of the chains were used. The model was run twice to test the general stability of the solution from the Markov chain. In addition, we estimated the time since the population had started to decline (t_a) with $t_a = t_f * N_0$ and N_0 corresponding to the estimated population size, as well as the ancestral population size (before the decline), with $N_1 = N_0/r$.

2.3.7. Estimating nuclear DNA diversity, Hardy Weinberg equilibrium and linkage disequilibrium

Based on the 15 loci genotypes, we ran population genetic analyses using the softwares GENEPOP version 3.4 (Raymond and Rousset, 1995) and GENETIX version 4.02 (Belkhir et al., 1996–2004). Nuclear genetic diversity was measured as the number of alleles per locus (A), the observed heterozygosity (Ho), as well as Nei's unbiased expected heterozygosity (He) (Nei, 1978). Deviations from Hardy–Weinberg equilibrium were tested using an exact test. For loci with more than four alleles, a Markov chain was used to obtain an unbiased estimate of the exact probability. The Markov chain was set to 100 batches, with 5000 iterations per batch and 10 000 steps of dememorization. Global tests across loci for heterozygote deficiency and heterozygote excess and pairwise tests for linkage disequilibrium were performed using Fisher's method (Sokal and Rohlf, 1994) with 10,000 batches and 10,000 iterations per batch.

2.3.8. Comparing genetic diversity with other brown bear populations

We compared the genetic diversity of the Deosai population with the one from other documented bear populations in Europe and North America (A, Ho and He when available). However the values given in the literature cannot be compared directly with our data as they do not represent the same number of individuals and the same set of loci. Consequently, we took the opportunity of having the whole dataset from the Scandinavian brown bear population (Bellemain, 2004) for a comparison based on the same number of individuals and the same loci. A random selection of 28 bears, in each of the 3 subpopulations of the Scandinavian genetic dataset (M, N and S; Waits et al., 2000), was repeated 1000 times to estimate genetic diversity (A, He, Ho) and compare it with the corresponding values in the Deosai population.

2.3.9. Assessing relatedness

Based on the 15 loci genotypes of the different individuals identified in the population, we calculated pairwise genetic relatedness between pairs of individuals using Wang's estimator (Wang, 2002) and the software SPAGeDi version 1.0 (Hardy and Vekemans, 2002). This estimator includes (1) low sensitivity to the sampling error that results from estimating population allele frequencies; and (2) a low sampling variance that decreases asymptotically to the theoretical minimum with increasing numbers of loci and alleles per locus (Blouin, 2003). Relatedness values range from 1 to −1, indicating the percentage of alleles shared among individuals. Theoretically, a value of 1 means that genotypes are identical; a value of 0.5 indicates that 50% of the alleles are shared (e.g. parent/offspring or siblings relationship). Unrelated individuals have relatedness values ranging from 0 to −1 with the more negative values indicating greater differences in the genotypes of the individuals. We also used the genetic dataset for the Scandinavian subpopulations (M, N and S) to compare the level of pairwise relatedness between the Deosai population and those 3 subpopulations (using the same loci).

3. Results

3.1. Individual identification, probability of identity and reliability of the data

Totally, 136 samples were collected and 63 (~46%) of those samples were successfully amplified for 4–7 loci (including the sex locus). Twenty-three samples were from females, 37 from males and the sex could not be determined for three samples.

The data were judged to be reliable due to a high global quality index among successfully amplified samples (Fig. 2). Nine samples were discarded for further analysis due to their low quality index (below 0.5; Fig. 2). Finally, 54 samples typed for 6–7 loci were considered. Among those 54 samples, 28 individual genotypes were obtained (16 males, 10 females and 2 individuals of unknown sex). Each multilocus genotype was found from 1 to 5 times, with a mean of 2.22 ± 1.08 (SE) times. One sample for each of the 28 genetically identified individuals was further typed with 9 more microsatellites. The mean quality index per sample was 0.85 ± 0.13 for the 54 samples typed using 6 microsatellite loci and 0.91 ± 0.10 for the samples typed using 15 microsatellite loci.

Age of the feces was estimated for all but 11 samples. There was a significant negative correlation between the age of fecal samples and the proportion of positive amplification (Spearman's $\rho = -0.279$; $p = 0.01$) (Fig. 3) as well as between the age of fecal samples and the quality index (Spearman's $\rho = -0.271$; $p = 0.02$).

The probability of identity among the six amplified microsatellite loci for unrelated individuals was 1.881e−05 and 1.206e−02 for related individuals (sibs), thus we could identify each individual reliably. The probability of identity among the 15 amplified microsatellite loci unrelated individuals was 5.827e−10 and 1.329e−04 for related individuals. This allowed us to perform reliable parentage and relatedness analyses.

3.2. Estimating current population size

The population size estimates varied depending on the rarefaction equation used. The Kohn's estimate yielded a population size of 47 bears (95% CI: 33–102), whereas the Eggert's estimate gave a size of 32 bears (95% CI: 28–58).

3.3. Investigating the signature and age of the bottleneck

The analyses of the population's expansion and decline using MSVAR, based on the exponential growth model (Beaumont, 1999) gave the following values: $\log_{10}(r) = -2.423$, $\log_{10}(t_f) = 0.297$, $\log_{10}(\theta) = -1.410$. The low r value ($r < 1$) implies that the original population size declined to current population size. Considering the mean population size estimates for each rarefaction equation (see above), the number of generations

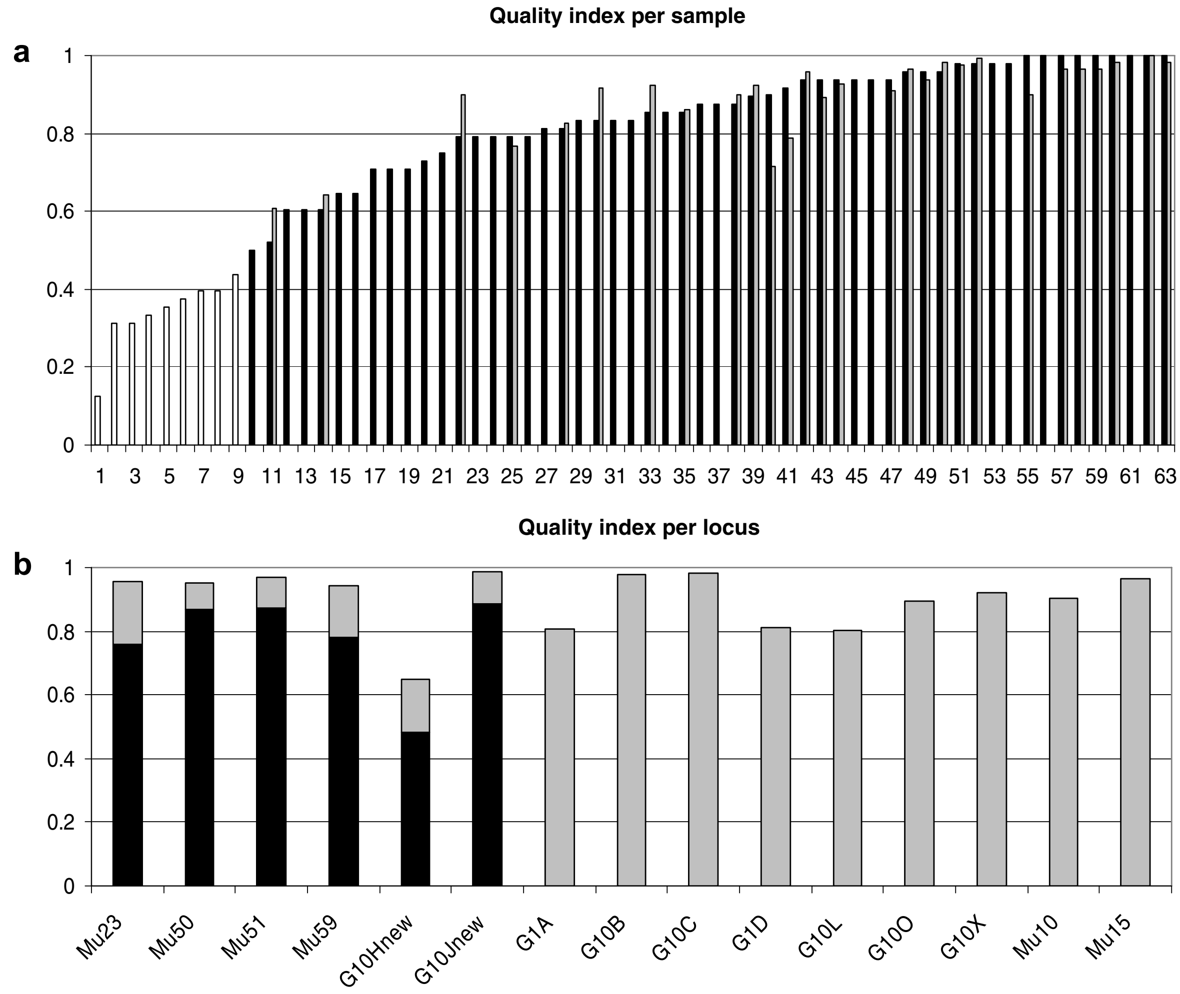

Fig. 2 – Quality indices (QI) per sample (a) and per locus (b) for successfully amplified genetic samples from brown bears in Deosai National Park, Pakistan. Black bars indicate QI for samples typed with 6 loci (for individual identification), grey bars indicate QI for samples typed with 15 loci (further analysis) and white bars indicate samples discarded from the analysis (because of their low QI).

since the population started to decline (t_a) was estimated to be between 63 and 93 and the ancestral population size (*N*1) ranged from 8000 to 11,750 individuals.

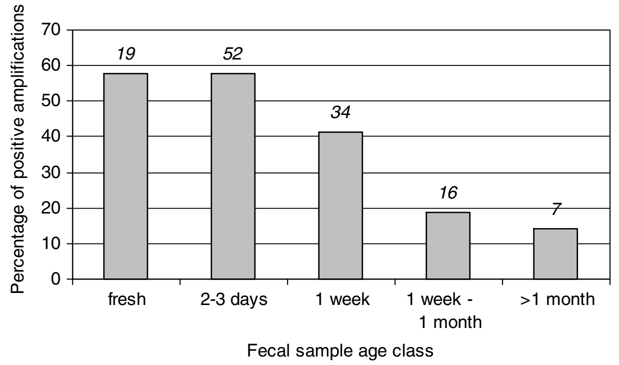

Fig. 3 – Success of brown bear fecal DNA amplifications from Deosai National Park, Pakistan, according to the age class of the fecal samples. Numbers above the bars, represent the sample size of each age class.

3.4. *Nuclear DNA diversity, Hardy–Weinberg equilibrium and linkage disequilibrium*

The number of alleles per locus among the 28 individual genotypes ranged from 2 to 7, with an average of 3.87 ± 1.36 (Table 1). The mean observed heterozygosity was 0.557, a value not significantly different from the unbiased expected heterozygosity (0.548). Global tests showed that the population is in Hardy–Weinberg equilibrium, although three loci (G10L, G10O, Mu10) had a significant deficiency in heterozygotes at the $p < 0.05$ level (Table 1). The overall multilocus Fis value was −0.016. Statistical tests for linkage disequilibrium were computed for all pairs of loci, and 15 of 105 tests revealed significant results ($p < 0.05$).

3.5. *Comparing genetic diversity with other bear populations*

The level of heterozygosity in the Deosai bear population (*Ho* = 0.557) was lower than in other bear populations in North America that are considered to have a good conservation sta-

Table 1 – Nei's unbiased expected (He) and observed (Ho) heterozygosities, and deviation from Hardy Weinberg equilibrium by locus from fecal samples of brown bears from Deosai National Park, Pakistan

Locus	Alleles	Allelic frequencies	He	Ho	*P*
Mu23	136	0.232	0.770	0.893	
	140	0.339			
	144	0.161			
	146	0.054			
	150	0.214			
Mu50	92	0.643	0.541	0.571	
	94	0.125			
	96	0.036			
	100	0.196			
G10B	136	0.382	0.466	0.518	
	150	0.618			
Mu59	95	0.25	0.830	0.857	
	109	0.196			
	111	0.054			
	113	0.089			
	115	0.036			
	117	0.214			
	119	0.161			
G10Jpak	80	0.518	0.656	0.678	
	84	0.089			
	86	0.232			
	88	0.161			
G1D	171	0.17	0.642	0.679	
	175	0.038			
	177	0.302			
	179	0.491			
Mu51	119	0.714	0.425	0.50	
	121	0.268			
	127	0.018			
G10Hpak	241	0.442	0.602	0.76	
	243	0.115			
	245	0.423			
	249	0.019			
G1A	189	0.593	0.496	0.5	
	191	0.019			
	193	0.389			
G10C	104	0.4	0.492	0.518	
	108	0.6			
G10L	143	0.204	0.773	0.583	0.009
	155	0.224			
	157	0.286			
	159	0.265			
	163	0.02			
G10O	193	0.019	0.037	0.037	
	195	0.981			
G10X	142	0.849	0.281	0.115	0.023
	154	0.057			
	156	0.057			
	158	0.038			
Mu10	140	0.094	0.656	0.5	0.0002
	142	0.057			
	150	0.019			
	152	0.434			
	154	0.396			

(*continued on next page*)

Table 1 – *continued*

Locus	Alleles	Allelic frequencies	He	Ho	P
Mu15	137	0.018	0.527	0.556	
	139	0.473			
	141	0.509			
Average			0.548	0.557	

Only significant P-values are shown ($P < 0.05$).

tus ($Ho = 0.78$ in North America; Paetkau et al., 1998 and $Ho = 0.66–0.76$ in different regions of Canada and USA; Waits et al., 1998). However, it is comparable to the level of heterozygosity in the Yellowstone area ($Ho = 0.55$; Paetkau et al., 1998) and higher than the level observed in some isolated populations such as the Kodiak Islands in Alaska ($Ho = 0.26$; Paetkau et al., 1998) or the Pyrenees in France ($Ho = 0.39$; Taberlet et al., 1997).

In comparison with each of the three subpopulations in Scandinavian bears, Deosai bears had a significantly lower number of alleles and observed and unbiased expected heterozygosity (for the same number of individuals and loci subsampled; Table 2). When compared to the mean genetic characteristics in the entire Scandinavia, the expected heterozygosity in the Deosai population is reduced by 17.5% and the number of alleles per locus by 44%.

3.6. Assessing relatedness

The average pairwise relatedness in the Deosai bear population was 0.0265 ± 0.292 (SE). This was not significantly different from the average pairwise relatedness in the subpopulations of the Scandinavian bears for the same loci (paired t-tests for each subpopulation: N: $r = -0.0232 \pm 0.044$; $p = 0.231$; S: $r = 0.015 \pm 0.044$; $p = 0.206$; M: $r = -0.001 \pm 0.032$; $p = 0.052$).

4. Discussion

4.1. Quality of the genetic data

We ensured a high reliability of the genetic data by repeating amplifications (multi-tubes approach) and selecting samples with high quality indices. The probability of misidentification was low, allowing us to identify unambiguously each individual. Therefore, we are confident that we have not overestimated the number of individuals in the fecal sampling.

The amplification success was correlated negatively with the age of fecal samples. Amplification success was relatively good (~58%) for fresh feces or feces that were only 2–3 days old and dropped to 41% for 1 week old samples, but this rate might still be acceptable. However, samples older than one week had a poor amplification success. We recommend, for future studies in Deosai, that fecal samples older than one

Table 2 – Comparison of the genetic diversity of brown bears between the Deosai population in Pakistan and the three subpopulations in the Scandinavian genetic dataset (mean over 28 randomly and repeatedly chosen individual bears)

	Pakistan			Scandinavia South			Scandinavia Middle			Scandinavia North		
	A	He	Ho	A	He	Ho	A	He	Ho	A	He	Ho
Mu23	5	0.77	0.89	7	0.70	0.73	7	0.82	0.83	6	0.72	0.70
Mu50	4	0.54	0.57	7	0.74	0.72	7	0.79	0.76	9	0.71	0.69
Mu51	3	0.43	0.50	7	0.78	0.80	8	0.77	0.75	8	0.76	0.74
Mu59	7	0.83	0.86	10	0.76	0.77	11	0.83	0.86	11	0.83	0.83
G10Jnew	4	0.66	0.68	6	0.57	0.58	6	0.66	0.66	7	0.75	0.75
G10Hnew	4	0.61	0.76	8	0.59	0.58	8	0.53	0.47	11	0.74	0.74
G1A	3	0.51	0.50	6	0.63	0.69	5	0.71	0.70	7	0.67	0.63
G1D	4	0.64	0.77	7	0.61	0.59	5	0.66	0.65	8	0.74	0.79
G10B	2	0.48	0.52	5	0.69	0.68	8	0.64	0.69	8	0.74	0.70
G10C	2	0.49	0.52	5	0.69	0.66	5	0.67	0.69	6	0.68	0.68
G10L	5	0.77	0.58	7	0.77	0.79	7	0.69	0.63	8	0.81	0.74
G10O	2	0.04	0.04	3	0.38	0.38	3	0.36	0.36	3	0.12	0.12
G10X	4	0.28	0.12	4	0.54	0.56	5	0.65	0.62	7	0.54	0.53
Mu10	5	0.66	0.50	8	0.80	0.79	8	0.74	0.75	8	0.78	0.75
Mu15	3	0.53	0.56	4	0.66	0.66	4	0.53	0.50	5	0.51	0.52
Mean	3.80	0.55	0.56	6.27	0.66	0.67	6.47	0.67	0.66	7.47	0.67	0.66
SD	1.37	0.20	0.24	2.07	0.11	0.11	2.07	0.13	0.13	2.07	0.18	0.17
P-values				6.82e−07	0.0121	0.059	1.02e−05	0.008	0.065	6.98e−07	0.0006	0.0161

P-values represent the significance of paired t-tests performed between the Pakistan population and each of the three Scandinavian subpopulations.

week not be collected in order to optimize the cost and benefit of the genetic analyses.

Brown bears in Deosai are mainly vegetarians (Schaller, 1977; unpublished data of fecal analysis). Previous studies have suggested that plant secondary compounds can inhibit PCRs (Huber et al., 2002). However, this study demonstrated that reasonable brown bear DNA amplification can be obtained from fecal samples composed mainly of plants (Murphy et al., 2003).

4.2. *The genetic status of the brown bear population in Deosai*

The analyses performed from the fecal DNA dataset allowed us to answer important questions regarding the management and conservation of bears in the Deosai population. First, the population size estimates provided by the two rarefaction indices are in the same order of magnitude as the numbers derived from the field censuses, which gives us confidence that those results are realistic. The census carried out during summer 2004 recorded 38 bears from the Deosai National Park, with a density of 19 bears per 1000 km^2 area (Nawaz et al., 2006). Based on this, the Eggert method seemed to underestimate the population size, whereas Kohn's method seemed to be more realistic, although the upper limit of the confidence intervals seems to be an overestimate. Unfortunately, the small sample size and small number of recaptures prevented us from using the MARK method, which is thought to give better estimates of population sizes (Bellemain et al., 2005). Considering the minimum number of individuals captured from the fecal samples (28) and the rarefaction method estimates, the field estimates appear to be conservative, though they fall within the range of the other estimates. Field methods usually give underestimates of wild populations, particularly for elusive animals (Solberg et al., 2006). The open terrain of the Deosai plateau, which allows bears to be observed, the small population size, distinctive marks on many bears, and the expertise that the field staff had gained over a period of 12 years from observing bears, probably contributed to the realistic observation-based estimates in Deosai National Park. We conclude that approximately 40–50 bears were present in the park in 2004.

The results from the analysis using the program MSVAR suggested that a decline in the Deosai population occurred approximately 63–93 generations ago using the mean estimates given by the rarefaction analysis and 80–100 generations ago, using a more realistic population size of 40–50 individuals. This period approximately corresponds to 800–1000 years ago, with a generation time of 10 years (calculated using the software RAMAS, Ferson and Akçakaya, 1990 and considering an age of first reproduction of 6 years old). The ancestral population (before the decline; N1) was estimated to contain 8000–11,750 individuals using rarefaction estimates or 10,000–12,500 individuals using a more realistic population size of 40–50 individuals. This estimate seems realistic considering an approximate area of 200,000 km^2 of bear distribution range in northern Pakistan and Kashmir, which gives a density of about 55 bears per 1000 km^2. These results suggest that the brown bear population in northern Pakistan might have undergone an approximate 200–300-fold decrease during the last thousand years. This decline cannot be linked to a single event or phenomenon. It was probably affected by both natural (climatic and geological) and socio-political factors. In the medieval warm period (1000–1200 AD), the bears certainly formed a single, large population, with a contiguous habitat in Hindu Kush, Karakoram and Western Himalaya ranges. The historic phase of glaciations in High Asia identified as a "little ice age" (1180–1840 AD; Kuhle, 1997; Esper et al., 2002; Mackay et al., 2005) is considered to have been similar in extent to the Neogeological stages (Meiners, 1997) and may have acted as a proximal cause of decline, destroying part of the population and fragmenting the rest. The influence of a growing human population, including large deforestation in the Middle Ages (Bertrand et al., 2002), political unrest due to presence of the Tibetan army in the area and its clashes with local people and China (Sheikh, 1998; Rashid S, personal communication) and the spread of firearms in the late 19th century, probably contributed further to the population decline and did not allow bears to colonize in a natural way.

Third, we assessed whether the Deosai population is currently at risk of inbreeding depression. The population genetics analyses revealed that the level of nuclear genetic diversity of the Deosai population is globally lower than brown bear populations considered to have a good conservation status, such as in Scandinavia or North America. In addition, and for the first time, we made an unbiased comparison of nuclear diversity between two populations, based on the same loci and same number of individuals. This analysis supports the conclusion that the Deosai population harbors significantly less heterozygosity and a smaller number of alleles per locus than any of the three subpopulations in Scandinavia. However, this population is in Hardy Weinberg equilibrium and its level of relatedness is similar to that in the Scandinavian brown bear population. Therefore, the Deosai bear population does not appear to be at immediate risk of inbreeding depression. Its level of genetic diversity is comparable to the brown bear population in the Yellowstone area, USA, which has become an isolated remnant, separated from other brown bears for nearly a century (Paetkau et al., 1998). A similar scenario could be envisaged for the Deosai brown bear, which probably lost genetic diversity due to isolation and genetic drift in the last centuries and due to the currently small population size.

Our final goal was to examine the degree of isolation of the Deosai population. Four individuals in our genetic dataset showed private alleles at two different loci, suggesting that they could be migrants (or descendants from migrants) from outside of the study area. Field observations support this hypothesis. Brown bears also exist in the Minimerg and Astore valleys, which are adjacent to Deosai National Park. Movements of bears have been observed between these areas during recent surveys, and the Deosai population may have interchanged not only with bears in these valleys, but also with the bear populations in the Neelam Valley and in Indian Kashmir through these valleys (unpublished data). When we began our studies of the Deosai brown bear population, we had expected to find genetic loss due to isolation and a small population; however, we documented a moderate level of genetic diversity. This strongly suggests that connectivity exists between the Deosai population and the neighboring populations through movements of individuals.

4.3. *Conclusions and recommendations*

We have documented that the Deosai brown bear population shows moderate levels of diversity and is not at immediate risk of inbreeding. The population probably began to lose genetic diversity about 1000 years ago, when it began to decline from a single large population throughout northern Pakistan. This resulted in fragmentation of the population into smaller units that lost connectivity during the course of time. The population decline stopped in Deosai about 15 years, ago when the population received increased protection. Under a scenario of an isolated population, the population would probably suffer from inbreeding today. Therefore, we believe that the moderate level of genetic diversity observed has been maintained by gene flow with adjacent populations in Pakistan and India. Nevertheless, this level of genetic diversity is lower than in healthy populations in Europe or North America. Maintaining and improving the connectivity with adjacent populations in Pakistan and India will be of paramount importance for the long-term survival of this small population in future.

We suggest that future studies continue to monitor the population carefully, both with field observations and genetic analyses. Concrete management actions should aim at maintaining and improving connectivity with other populations to maintain or improve levels of genetic diversity. Otherwise, the population will continue to lose genetic diversity over time. Increasing the size and range of fecal sampling would not only allow a more precise estimate of the population size, but also give a better estimate of incoming gene flow.

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Paper III

AN INCREASING LOW-PRODUCTIVE, HIGH-ALTITUDE BROWN BEAR POPULATION IN SOUTH ASIA; A SUCCESSFUL CASE OF NATIONAL PARK MANAGEMENT

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Abstract

We monitored a brown bear population in Deosai National Park (DNP) from 1993 through 2006, and documented population growth from annual counts and estimated reproductive parameters from recognizable bears. The observed population growth was 5% annually, which was a product of both reproduction and immigration. We documented an extremely low reproductive rate in the Deosai population, due to late age of first reproduction (8.25 years), a long reproductive interval (5.7 years), and a small litter size (1.33). The family association (4.2 years) is the longest ever reported for brown bears and might have contributed to higher survival of young. The reproductive rate of the Deosai population is the lowest among all documented brown bear populations. Poor habitat quality, low quality food, high seasonality, and extreme weather conditions in the Himalaya probably explain poor reproductive performance.

The recovery of the brown bear population in Deosai is significant, because the species is declining throughout most of their range in South Asia due to habitat loss and overexploitation by humans. Successful control of human-caused mortalities and participation of communities were the key factors toward this success. Strict law enforcement and surveillance of the park sharply reduced bear mortalities. Community participation, achieved by recognizing rights and introducing incentives, reduced resistance against the conservation efforts, reduced pressure in bear habitat and helped reduce poaching. The DNP is a rare example of successful cooperation between an NGO, people and the park management. Brown bear conservation efforts in South Asia must target reducing human-caused mortalities, particularly of adult females. Involvement of people can increase efficiency in conservation, in addition to reducing cost and conflicts.

Key words: demography, Pakistan, population growth, protected area, reproduction, *Ursus arctos*

Introduction

Protected Areas (PA) are considered to be vital for both biodiversity conservation and sustainable development, and more than 100,000 PAs have been created worldwide (2003 United Nations List of Protected Areas). The number of PAs has grown impressively in South Asia during the last five decades; with about 1500 sites on the UN List, covering 6.87% of the total land (Chape et al., 2003). However, rapidly increasing human populations and demands for natural resources have arrested the creation of PAs in a struggle between conservation and development. Ecologically sustainable development that accommodates economic and social needs of the society is the emerging perspective for PA management (Phillips, 1994; Sheppard, 2004). Resident communities are viewed increasingly as important stakeholders and their participation often is deemed crucial for the success of the PAs (Mishra et al., 1989; Dearden et al. 2005; Hiwasaki 2005), although their participation in itself does not ensure success (Oates 1995, 1999). This approach is very relevant in south Asian countries, where the livelihood of rural communities and PAs are essentially linked (Ghazali and Khairi, 1994).

In contrast, Pakistan's conservation policies and management strategies have changed little. Management of the > 200 PAs in Pakistan occurs without public participation and current legislation neither recognizes public rights nor allows resource utilization within protected areas. Confrontations with local communities, financial constrains, and poor management infrastructure contribute to the fact that most PAs are not functional. IUCN Pakistan (2000) developed a comprehensive action plan framework for strengthening the country's PAs and emphasizing community participation, but it largely remains to be incorporated into national policy.

Deosai National Park (DNP), in the Northern Areas of Pakistan, was created in 1993 (GoP Notification 1993). Alpine pastures are a rare and usually degraded resource in Northern Pakistan (Ehlers and Kreutzmann, 2000), where much of the landscape is just rock and ice. The vast grazing grounds of Deosai make a significant contribution to the livelihood of local and nomad communities. Fishing, falconry, and poaching of brown bears (*Ursus arctos*) for fat are other means of income. Because the wildlife legislation (Northern Area Wildlife Preservation Act 1975) does not allow any kind of resource extraction from a park, the new legal status of the area was not acceptable for the concerned communities. The Himalayan Wildlife Foundation (HWF), a nongovernmental organization that was instrumental in the creation of the DNP, took the case of Deosai as an opportunity to test emerging approaches towards PAs, where ecological sustainable rural development is linked with biodiversity conservation (Mishra et al., 1989). In 1993, HWF collaborated with the Northern Areas Forest and Wildlife Department and local communities on a conservation program based on three main principles: (1) recognition of community rights through a zoning plan of the park; (2) protection of biodiversity through a system of enforcement and monitoring; and (3) community involvement and assistance through (a) employing staff from neighboring villages, (b) developing ecotourism and training locals for various tourism-related services, (c) assisting communities and mobilizing resources for development projects, and (d) generating revenue and sharing it with the communities (see HWF, 1999a for details). The zoning plan allowed communities to continue utilizing pastures within specified areas of the park, but not in a core area for bears. This provision reduced the conflict with communities over park resources, and at the same time reduced human presence and grazing pressure in core bear areas. Principal (3) further catered cooperation and participation of the communities in park management. Monitoring and park management were completely integrated, as staff employed for law enforcement maintained permanent presence in the park, monitored individual bears and contributed most of the data. Their continuous patrolling in the study area was probably the major factor deterring poaching.

The primary objective of DNP was to protect a small population of highly threatened Himalayan brown bear (*U. a. isabellinus*); therefore its population size was set as an indicator of the park's success from the beginning (HWF, 1999a). Brown bears are found throughout most of the northern hemisphere and occupy a variety of habitats from tundra to temperate forests

(Servheen et al., 1999). Variation in energy and environmental conditions over a geographical range induces variation in life history (Rosenzweig and Abramsky, 1993), consequently life history traits in brown bear are diverse (Dahle and Swenson, 2003a; Stringham, 1990; Zedrosser, 2006). Habitat stability (i.e., the degree of its seasonality and predictability) and temporal stochasticity are the two environmental factors that have major impacts on life history (Clark and Yoshimura, 1993; Southwood et al., 1974). In environmental extremes (high seasonality, low productivity and temporal stochasticity), a conservative life-history strategy is expected (Boyce et al., 2002; den Boer, 1968). Deosai represents a highlands ecosystem (>3000 m, Mani and Giddings, 1980), characterized by unpredictable, unstable, highly seasonal, and extreme environmental conditions. The life history of high-altitude brown bears has never been documented. However brown bears living at higher latitudes in North America and Europe are known to be less productive (Ferguson and McLoughlin, 2000; Boyce et al., 2002; Swenson et al., 2007). High latitudes and altitudes are similar in environmental factors (e.g. thermic-seasonal events), though the latter have more severe conditions (Mani, 1990). The Deosai population might therefore be less productive than populations at lower altitudes. The reproductive performance and survival of individuals determine population growth (Schwartz et al., 2006). Because the Deosai population is small and facing threats like poaching and habitat loss, we considered it essential to document the population's rates of reproduction and mortality in order to formulate an appropriate management strategy for its long-term survival.

Our objectives were to (1) evaluate the effectiveness of park management in terms of the trend of the brown bear population and (2) estimate demographic parameters and factors affecting viability of high-elevation brown bears. Findings of this study can provide directions for the conservation of brown bears living elsewhere in high Asia.

Materials and Methods

Study Area

The DNP (75° 27' N, 35° 00' E) is a 1800 km^2 alpine plateau east of Nanga Parbat Peak, Northern Areas, Pakistan. Elevations range from 3,500 to 5,200 m, with about 60% of the area between 4,000-4,500 m. Mean daily temperatures range from –20°C to 12 °C. The annual precipitation is 510 mm to 750 mm, and falls mostly as snow (HWF, 1999a). Vegetation is predominately herbaceous perennials, grasses and sedges. The brown bear is the flagship species of the park; other mammals include Tibetan wolf (*Canis lupus chanco*), Himalayan ibex (*Capra ibex sibrica*), Tibetan red fox (*Vulpus vulpus montana*), golden marmot (*Marmota caudata*) and 17 other small mammal species. The Deosai Plateau (DP) is a typical highlands ecosystem, which is characterized by low atmospheric pressure, cold, aridity, low oxygen and carbon dioxide levels, intense isolation, rapid radiation, and high ultraviolet radiation (Mani, 1990; Mani and Giddings 1980). The area has been dynamic climatically and geologically during the late Holocene (Kuhle, 1997; Meiners, 1997).

The DP is a relatively flat area between narrow valleys and steep mountains, close to the Line of Control with India. Although there is no permanent habitation, because of the high altitude and extreme climate, there are many settlements along the periphery of DNP. They are located in numerous valleys and have various stakes in Deosai, especially traditional grazing rights. Their livelihood system is based on cultivation and livestock rearing, which is why the DP's alpine pastures are important. All but four peripheral communities utilize DP's outer slopes and peripheral valleys for grazing. Four communities, Sadpara, Shilla, Dhappa and Karabosh, claim traditional grazing rights within the DNP boundaries and their livestock occupy the eastern part of DP during summer. The total population of the peripheral communities is approximately 13,000, with about 25,000 livestock. In addition to these sedentary communities, there are nomad groups (*Bakarwals* or *Gujjars*), which come from the lowlands and compete for grazing resources. Approximately 9,000 livestock (belonging to resident and nomad communities), mainly goats and sheeps, grazed within the DNP in 2004.

Population Census and Monitoring:

Since the inception of the brown bear project in 1993, the HWF has operated a summer field camp in DNP from June to October, depending on snow conditions. The primary responsibility of the permanent HWF field staff was to observe individual bears regularly and document their movements and behavior. The treeless vegetation and relatively gentle terrain allowed for good visibility, which helped locate bears from a long distance (2-3 km), and permitted following them even without aided technology like telemetry. In addition, 7 individuals (3 males and 4 females) were immobilized and radio-collared (Telonics VHF transmitters) in 1996 and 1997 (HWF, 1999b, Nawaz et al., 2006). Field teams of 2-3 people followed individual bears, staying at a distance of about 1 km, for 1-7 days in each trip, making night stays in portable tents. Animal positions and movements were marked on field maps, and individuals' behaviors (activity pattern, interactions with other individuals, etc) were documented (Nawaz and Kok, 2004). These intensive surveys and long association with the bears, in addition to individual differences in markings, allowed field staff to recognize individuals. Individual recognition from morphology has been used in some other brown bear studies. Sellers and Aumiller's (1994) study of brown bear population at McNeil River, Alaska, was based on individually recognizable bears, and Craighead et al. (1995) also used some unmarked brown bears in their analysis, assuming them to be recognizable. Smith (1991) reported morphological and behavioral characteristics to discriminate between sexes in a guide for male-selective grizzly bear hunting.

In Deosai the following factors helped in individual identification:

(1) Color variation: Variation in pelage color has been documented in Himalayan brown bears (Sterndale, 1884; Schaller, 1998) and in DNP four pelage colors were identifiable; blonde,

silvertip, light brown and dark brown. Individuals generally darkened with age, and females were usually lighter than males.

(2) White patches: Many individuals had characteristic white patches. These patches were variable; some individuals had a white snout, others white ear tips. White oval patches on the shoulders were relatively common, but their sizes were variable. Some individuals had small white marks on the shoulders, some had completely white shoulders, and in some individuals a large white patch covered bothshoulders, lower parts of the neck and some parts of the chest.

(3) Size: Brown bears are sexually size dimorphic (Schwartz et al., 2003b). Adult females in Deosai have a mass of 60-80 kg, adult males 120-150 kg, and subadult males 50-60 kg. Sex determination in subadults was relatively difficult, until females gave birth.

(4) Radiotelemetry: The 7 radio-collared adults comprised about 40% of the adult population at that time. This increased the reliability of the observational study.

(5) Genetic analysis: A genetic analysis of the population based on fecal samples was conducted in 2005, which gave a population estimate similar to the results of the field census (Bellemain et al., 2007). The genetic analysis verified maternal relationships among individuals that were assumed from field observations, and also verified patterns of individuals' distributions as observed in the field.

(6) There was little turn over of the field staff, allowing people to remain associated with the project throughout this study. Their personal experiences and ability to recognize individuals were valuable for the quality of the data.

This study particularly targeted females with young, which allowed documentation of the females' reproductive activity and survival of young. In addition the entire park was surveyed every year during 10-15 days in late September or early October to obtain a population census. The DNP was divided into five blocks, and line transects were placed in each block to cover most of the park. This end-season population census allowed us to document individuals that could not be observed during the summer season. If a new individual was found during the census and we were not sure about its identification, it was treated as an immigrant. Therefore the population census was comprised mainly of identifiable individuals.

We estimated age using the size of individuals if they had not been monitored from young ages. We identified three classes of sizes in independent bears; small, medium, and large. Small bears were considered to be subadults (5-7 years old), and medium and large size bears were considered to be adults.

Estimating Reproductive Parameters

To determine the age of primiparity, we used observations of females that were monitored from birth, except for one which was followed from an estimated age of 4, when she arrived with an immigrant mother and separated from her that year. About 80% of the young in this area separate from their mother at the age of 4 years (see results). We accounted for the loss of some nulliparous females (emigrated or died) when calculating the mean age of reproduction (Garshelis et al., 1998). This method gives an unbiased estimate of the mean age of primiparity. One 9-year-old nulliparous female did not produce a litter by the end of the study; it was treated as having produced the next year (Garshelis et al., 1998), because the maximum observed age of primiparity was 8 years. We used bootstrapping (Efron and Gong, 1983) to estimate standard error in the statistical package R 2.4.1 (R Development Core Team, http://www.r-project.org).

We estimated the litter interval and length of family association, correcting for incomplete intervals, by using a method analogous to Garshelis et al. (1998). As each female can have multiple litter intervals, each litter interval was used as sample unit to calculate the reproductive interval (Garshelis et al., 2005). The monitoring of 16 females allowed us to calculate litter interval, 9 of them were monitored from 1993. The average monitoring period for these 9 females was 11 years (range: 5-14), and these females provided some complete intervals. The 7 other females were monitored from 1998 and 2001, with an average contact period of 4 years (range: 2-7 years). These females mostly gave open ended intervals and the Garshelis et al. (1998) method allowed us to use these data.

Family association is the time between a birth to successful separation of a litter and is important, because it influences reproductive interval, and because brown bears do not breed until they have separated from their young (Dahle and Swenson, 2003b). Each litter was taken as a sampling unit. If a cub-bearing female was lost from contact before family breakup, the data were used up to that point.

We calculated mean litter size using all litters observed after den emergence. We used two methods to calculate reproductive rate (young born/ year/ reproducing adult female). 1) by dividing the mean litter size by the mean litter interval. 2) We used the reproductive history of 6 females that provided 11 complete birth intervals, and calculated mean reproductive rate (*m*) using the following equation (Hovey and McLellan 1996):

$$m = \frac{\sum_{i=1}^{n} \frac{\sum_{j=1}^{p} L_{ij}}{\sum_{j=1}^{p} B_{ij}}}{n} \quad ,$$

where *j* is an observation of paired litter size (*L*) and litter interval (*B*) from the reproductive history of female *i*, *p* is the number of observations of *L* and *B* recorded for female *i*, and *n* is the total number of females. The average monitoring time for these females was 11.5 years (range: 7-14), and *p* values ranged from 1-3 per female.

Estimation of Survival Rates

We determined survival of cubs-of-the year ("cubs") and yearlings by following their mothers. This method has been used in American black bears (*Ursus americanus*) and brown bears (Doan-Crider and Hellgern, 1996; McLellan et al., 1999; Schwartz et al., 2006; Schwartz et al., 2003b; Swenson et al., 2001). Survival was estimated by dividing the number of young surviving to the next year by the total number of young in an age class. Some females and associated young disappeared from the study area during the winter, and we were not sure about the fate of the associated young. We made two data sets to deal with them; in one data set we censored (C) these young, and in the second data set, they were assumed dead (AD) (Haroldson et al. 2006). We reported survival in a range between $\bar{S}_C$ and $\bar{S}_{AD}$.

For age groups ≥2 years, estimating mortality rates was more difficult, because, in addition to known mortalities, many individuals were lost from contact. Known mortalities were all illegal shootings; we collected remains of shot bears and in some cases hunters were prosecuted. A bear that was followed during previous years and was not observed throughout a summer season without any indication of its death was treated as an "undocumented loss". There could have been three possibilities concerning fate of such an undocumented loss ; 1) death, 2) emigration, 3) they have home ranges partially outside DNP and did not visit, or were not detected in, DNP every year. "Immigrants" were all new individuals observed after the first year of study; these could have been individuals coming from neighboring populations or individuals that visit Deosai occasionally. All new individuals were treated as immigrants, unless we were very sure about their identification. These new individuals were given a new ID and monitored until they were lost from contact or the study ended. We maintained visual contact with the individuals monitored since 1993 during most of the study period.

We treated known mortality as the minimum mortality rate, and the total loss (including undocumented loss) as the maximum mortality rate, and estimated survival for both cases as (Eberhardt et al. 1994):

$$S = 1 - \frac{recorded\ deaths}{bear\ years\ observed}\ .$$

Estimation of Population Growth

We estimated the finite rate of increase (λ) from annual censuses of the Deosai population, with λ as the ratio of numbers in two successive years (Caughley, 1977). The λ was calculated by the exponential rate of increase, β, which was estimated by regressing population size (ln N) on year. We ran this model in MINITAB software (MINITAB Release 14.20, 1972 - 2005 Minitab Inc.). We observed higher counts in the last 3 years of the study. In order to disassociate the impact of these years on overall population growth, we calculated another regression model excluding these last 3 years.

We have documented an exchange of individuals with adjoining populations (Bellemain et al., 2007), which might have influenced the growth rate. In order to estimate the intrinsic growth of the population, we used the deterministic Leslie matrix (Leslie 1945, 1948) in PopTools (http://www.cse.csiro.au/poptools/). We used 30 age classes and the postbreeding census (reproductive rates were multiplied with survival rates). The dominant eigenvalue of the Leslie matrix gives population growth rate (λ). We calculated λ for the best and worse case scenarios, using minimum and maximum mortality rates, respectively. We also calculated elasticity, which measures the percentage change in λ due to percent change in mortality or fecundity (Stearns 1992).

Small populations are vulnerable to genetic, demographic and environmental stochasticities (Soule´, 1987), and these stochastic events can depress population growth (Lacy 2000). We conducted a Population Viability Analysis (PVA) in Vortex 9.61 (Lacy et al., 2006), which allows assessing impacts of stochasticity. The difference between the deterministic growth rate and the simulated growth rate provided an indication of stochastic impacts. Demographic stochasticity is the random fluctuation in birth and death rates and sex ratio of a population. Vortex models annual variation in births, deaths and sex determination as binomial distributions and generates pseudo-random numbers. Environmental stochasticity is the fluctuations in the probabilities of birth and death from environmental variation, and Vortex model it as a normal distribution (Lacy et al., 2006). We did not model genetic impacts because the Deosai population is not facing inbreeding depression (Bellemain et al., 2007). In Vortex we simulated Deosai population by 1000 iterations, using base parameters as; age of primparity: 8 years, maximum breeding age: 30, %adult females breeding: 17.54 (1/litter interval), distribution of litter size: 1 = 70% and 2 = 30%.

Comparisons with other Brown Bear Populations

We compared reproductive parameters of the Deosai population with North American and European populations. Reproductive data from other Asian high-altitude brown bear populations are not available; however high altitude environments have some similarities to that of high latitudes (Mani, 1990). Demography of brown bear populations has been reported to be influenced by high latitudes (Ferguson and McLoughlin, 2000), and we therefore emphasized comparisons with high-latitude populations.

Results

During 14 years (1993-2006), 86 individuals were followed for 423 bear-years, with 24 females, 18 males and 44 young (up to 4 years of age), monitored for 169, 147, and 107 bear-years, respectively. Twelve females were monitored for more than 3 years; their collective observation period was 107 bear-years. The radiotelemetry sample consisted of 3 males, 3 females with 4 dependent young, and one lone female, with a collective monitoring period of 20 bear-years. The mean monitoring period for adult bears was 6.4 years (SD: 4.8), ranging from 1 to 14 years.

Population Size

We counted 19 individuals during 1993, including 7 males, 7 females, and 5 young. Annual censuses in the subsequent years showed a gradual increase, with a minimum population size estimate of 43 individuals towards end of the study (Fig. 1 and 2). In 2006, there were 17 males, 15 females and 11 young in the population. Averaged over the study period, there were 41% adults, 8% subadults and 18% young (up to 4 years of age) in the population (Fig. 1). The adult sex ratio remained quite equal, except for recent years when it became male biased. However, a 14-year mean of the population sex structure showed sexes at parity; the female to male ratio was 1:1 (SD: 0.17) (Fig. 1). Among the 11 cubs that successfully grew to adults during the study period, the female to male ratio was 6:5.

The current population density within DNP was about 24 bears per 1000 km^2, assuming that the bears only used DNP, or 13 per 1000 km^2, if we included an area 1400 $km^2$of surrounding valleys, which was also part of the bears' home ranges. The density was not uniform. Therefore the high-density area between Shatung and Shingo-Shigar rivers and adjacent valleys (Shilla to Karabosh) was designated as the park's core area (HWF, 1999b). A rugged area in the center of DNP, termed by the HWF team as "Black Hole", had an especially high density, seasonally as high as ~1 bear/km^2. The higher density in Black Hole occurred during the summer and was probably related to higher biomass production, ruggedness, and absence of human structures (camps, roads, etc) (Nawaz and Swenson, unpublished).

Reproductive Parameters

We observed 9 nulliparous females in our study sample, but included only 6 of them with reliable age estimates. Only 3 nulliparous females produced litters during the observation period, the other 3 were censored from the sample before giving birth. No female in our observation produced cubs before 7 years of age, and the mean age of reproduction was 8.25 (range: 7-10, Table 1). One young nulliparous female was radiocollared in 1996 and monitored for 9 years (3 years with radiotelemtry and thereafter with visual observations) before we lost contact at an estimated age of ≥13 years. This female was never observed with a litter during the observation period and was one of the females of unknown age excluded from the sample.

The litter interval was calculated based on 24 observed intervals, 11 closed and 13 open-ended, for 16 females. Among the closed intervals; 3 belong to one female, 2 to 3 females, and 1 each to 2 females. The mean litter interval was 5.7 years (range: 4-8, Table 1). We observed 44 cubs in 14 years, and documented the successful weaning of 11 young; the rest were lost from contact. The mean length of family association was 4.2 years (range: 2.5-4.5, Table 1).

We observed 44 cubs in 33 litters from 22 females. There were 4 litters from 1 female, 3 litters each from 3 females, 2 litters each from 3 females, and the remaining 15 litters were produced by individual females. Litters consisted of 1 or 2 cubs, and averaged 1.33. The proportion of two-cub litters was 0.3.

Both methods produced similar estimates of reproductive rate (natality), as 0.233 and 0.234 (SD: 0.066) cubs per adult reproducing female per year, by method 1 (dividing litter size by litter interval) and 2 (using reproductive history of females), respectively.

Survival Estimates

During the study period, the total number of known immigrants to the population was 41 and total loss of individuals either by known human-caused mortalities or for unknown reasons (emigration, mortality, etc) was 37. Eleven males, 11 females with 14 dependent young (12 cubs-of-year, 2 yearlings), and 5 lone females came to Deosai from neighboring areas, an immigration rate of 2.9 individuals per year. We do not know the proportion of mortalities in the undocumented loss of individuals. However 24% of the total loss was known human killings and was comprised mostly of adult bears (78%). The population gained more males than it lost (10 vs 3), which likely contributed to a higher population of males in recent years. Unlike immigrant males, which kept arriving to Deosai over the time, about 50% of the immigrant females left Deosai (lost from visual contact) within 3 years. Similarly, 4 resident females were out of contact for part of their monitoring period; 1 for 1 year, 2 for 2 years and 1 for 3 years. This observation suggests that either the DNP is only part of some individuals' home ranges, or that some bears shift home ranges periodically.

We knew of few mortalities of cubs and yearlings; only 1 cub was known to be shot illegally with its mother. The others either survived to the next age class (81%, n: 69) or were lost from our visual contact (17%). The minimum annual mortality of the $\geq$2 age group was 2.35% (considering only known cases of deaths) and the maximum was 7.62% (including all undocumented loss) (Table 2).

Population Growth

The regression model fit the data well (R^2: 0.867), and the slope (β) was positive and statistically significant (F: 56, P = 0.00), suggesting growth in the population (Fig. 2). The estimate for the parameter β was 0.051 (SE: 0.0058), which corresponds to a finite growth (λ) of 1.05, or 5%, per year. The 80% and 95% confidence intervals for λ were (1.04, 1.06) and (1.03, 1.07), respectively. The regression without including the data from the last 3 years also showed a significant growth (λ: 1.036, F: 30.84, P = 0.00), suggesting that population growth is not just driven by counts in recent years. Thus, the population doubled from 1993 to 2006.

The deterministic estimates of intrinsic λ by the Leslie matrix and Vortex methods were similar under both best-case and worst-case scenarios (Table 2), and stochastic variations did not produce a large difference in λ. Population growth was highly sensitive to survival rates; the stochastic estimate of λ under the best-case scenario was 1.030 (95% CI: 0.968-1.093) and declined to 0.965 (95% CI: 0.794-1.135) when undocumented loss was treated as deaths. The elasticity of λ to survival declined gradually with age. Age groups 1-7 (prior to age of reproduction) produced the highest elasticity (0.0601 each), which was 1.3, 3.5 and 8 times higher than the survival elasticity of age groups 10, 20, and 25, respectively. The relatively large difference between intrinsic population growth rates estimated under best- and worst-case scenarios (0.965-1.030) indicates uncertainty in the intrinsic population growth. However the population would be intrinsically stable only if at least half of the undocumented loss actually survived (λ: 0.997 at 50% survival of undocumented loss).

Discussion

Comparison with other Brown Bear Populations

The reproductive parameters of 35 brown bear populations (30 North American populations (Mclellan, 1994; Case and Buckland, 1998; Garshelis et al., 2005; Kovach et al., 2006; Schwartz et al., 2006), and 5 European populations (Frković et al., 1987; Sæther et al., 1998; Frković, 2001; Swenson et al., 2001; Zedrosser et al., 2004), range between 3-9.6 years, 1.4-2.5 cubs, 2.4-5.8 years, and 0.36-0.96 cubs/year/adult female for age of first reproduction, litter size, reproductive interval and reproductive rate, respectively. The eight North American high-latitude populations (Table 3) are less productive than other terrestrial and coastal brown bear populations (Ferguson and McLoughlin, 2000). High-latitude populations have especially delayed age of reproduction (6-9.6 years). The reproductive parameters of the Deosai population are much lower than these other low productive populations. The Eastern Brooks Range, Alaska is the least productive population in North America, but this is 1.8 times more productive than the Deosai population. At the other end of the spectrum, the Scandinavian population has a reproductive rate that is 4.2 times higher than in Deosai.

Among 21 populations (from above-cited sources reporting cub survival), cub survival range from 0.34 to 0.96. The average yearling survival range was 0.58-0.97 in 15 brown bear populations. The cub (0.800-0.965) and yearling (0.848 -1.00) survival in Deosai population is therefore among the highest reported for brown bears.

Life-history Strategy

Bunnell and Tait (1981) suggested that nutrition is the primary factor regulating reproductive parameters in bears. The Himalayan brown bear is predominantly vegetarian with a low meat content in its diet (unpublished data based on scat analyses and hair isotope analyses). The dietary meat content and body mass (which is also linked to nutrition, Hilderbrand et al., 1999) are important indicators for reproductive success and mean litter size in brown bears (Hilderbrand et al., 1999, Dahle et al., 2006). Moreover, at high altitudes available resources and energy intake are low and cost of metabolism is higher (Mani, 1990; Westerterp and Kayser, 2006). Therefore, the constrains of high altitude environment (low productivity, high seasonality, high cost of living) together with low dietary meat and relatively high cost of nursing in brown bears (Farley and Robbins, 1995), have probably reduced flexibility in the life history traits by inducing limits on maximum performance. These limits on life history can be explained by considering physiological thresholds. For instance age of first reproduction depend on the threshold of a female's body mass and size (Bunnell and Tit, 1981; Blanchard, 1987; Garshelis et al., 1998), whereas litter size is related to female condition (Craighead et al., 1995; Hilderbrand et al., 1999).

The litter interval depends on the family association, because female brown bears do not reproduce before young are weaned (Schwartz et al., 2003a). The family association is influenced by numerous factors like condition of mother and offspring, and availability and quality of food resources (Bunnell and Tait, 1981; Craighead, 1995; Dahle and Swenson 2003a). From this conditional model we expect a longer family association from a female in poor condition living in a low quality habitat. The long family association has an energetic cost due to prolong nursing (Hilderbrand et al., 2000), as well as a reproductive cost. The theories of parent-offspring conflict (Trivers, 1974) and intergenerational trade-off (Stearns, 1992) predict increased offspring fitness with this maternal investment, with the following suggested benefits to the young: (1) maternal care increases growth of offspring, and in brown bear this effect is more pronounced in smaller litters (Dahle and Swenson, 2003a), (2) size at weaning is related to survival and reproduction in mammals, including brown bears (Zedrosser et al., 2006), and (3) long family association may reduce mass loss during hibernation (Dahle and Swenson, 2003a).

Small litter size reduces reproductive output, but increases survival of young because cubs are larger in small litters (Dahle and Swenson, 2003a), and survival is related to size (Dahle et al., 2006; Zedrosser et al., 2006). Another advantage of small litter size is a lower cost of reproduction and nursing for females, and may have a positive influence on future reproduction of females (Stearns, 1992), particularly in a low-productive environment.

The life-history strategy in the Himalayan brown bear, primarily induced by constrains of the environment and low nutrition, may have selective advantages in high-altitude environments, because low fecundity increases the population's ability to persist in stochastic environments

(Demetrius, 1975; Murdoch, 1966). The relatively low impact of stochastic variation on the estimate of λ in the Deosai population (indicated by a small difference between the deterministic and stochastic estimates of λ) is probably due to the low reproductive rates, and supports this conclusion. In this low-productive strategy, females allocate resources in a less productive but safer way, therefore spreading risks of reproductive failure (Ferguson and McLoughlin, 2000), and increasing the geometric mean fitness of the population (Yoshimura and Jansen, 1996).

Meeting the Management Goal

The documented statistically significant population growth during the study period showed that DNP had met its primary goal. The observed rate of population increase (λ: 1.05) was higher than the calculated intrinsic growth (0.0965-1.030), which implies that the park also has provided a refuge for bears from adjoining areas. We do not know about the status of bears in surrounding areas, whether DNP served as a magnet, resulting in a lower density around DNP, or whether DNP received a dispersing surplus from surrounding areas. Population growth is sensitive to survival and our survival estimates were in a range between minimum and maximum. The contribution of reproduction to the observed growth is difficult to interpret, because we cannot resolve the proportion of mortalities in the undocumented loss. However, even in the best case scenario, the 95% CI on the intrinsic λ bounds 1, indicating uncertainty in intrinsic growth potential at all levels of mortality. Uncertainty about estimates of population growth is a general problem in brown bears, because even healthy populations only achieve modest rates of increase. Consequently, confidence intervals around λ for a growing population typically overlap 1, especially for small populations where sample size is always small. However, as indicated by Schwartz et al. (2006), even with this uncertainty, other evidence must be considered when evaluating the overall success of a program. In the case of the Deosai brown bear population, the preponderance of evidence suggests that our program has been successful and that the park bear population has increased.

The DNP had a three-fold challenge for management since its inception; a biological challenge to conserve the small brown bear population, a resource management challenge to balance the needs of people without compromising ecological integrity, and a sociopolitical challenge to build the confidence of the local communities and engage them in conservation. The key factors behind the success of the park appear to be the control on human-caused mortalities and community participation. The support of the local communities, a vigilant monitoring system, and cooperation between military, police and forest departments have contributed to reducing mortalities. The main entry points to the park were guarded by the staff of the police and the forest department, and all people entering or leaving the park were checked carefully. Frequent patrolling throughout the park helped to identify any illegal human presence within the park and surrounding valleys. Poachers were arrested and prosecuted, and one military officer was punished in court-martial for a violation in the park. This strict law enforcement, in addition to awareness campaigns, greatly reduced bear poaching incidences, which was a big problem in past, as suggested by the existence of a bear-parts market (Nawaz, 2007).

Community participation was achieved by recognizing community rights and sharing park benefits, which was a major departure from the conventional PA management in Pakistan. The park started paying multiple benefits to the local communities. The park entry fee generates a considerable amount of revenue (about US$ 13,300 in 7 years, 2000-2006), national and international visitors to the park are increasing, resulting in increasing income from tourism-related services, and 18 people have been employed by the Forest and Wildlife Department. These benefits, coupled with provisions to allow communities to sustain their livelihood needs in a conservative way, have gradually reduced resistance against the park and negative attitudes towards bears.

Management Implications

The Deosai population is not isolated (Bellemain et al., 2007). The net influx of individuals occurred in two main phases; 1995-1998, and 2001-2004. The first was perhaps due to habitat improvement following zoning and decreased human access in the park. The second influx started in 2001 after the Kargil War, an armed conflict between India and Pakistan that started in 1999, and postwar development in the area (particularly construction of new paved roads). The Deosai population may be connected to the brown bear populations in Astore and Minimerg valleys, which in turn are connected with the Neelam Valley and the population in India (Nawaz 2007) (Fig.3). This movement between Deosai and adjoining areas has important implications for conservation, through maintaining gene flow and influencing demographic processes. The long-term viability of the Deosai and neighboring populations demands management on a broader landscape level. Because some individuals apparently have home ranges larger than the park, the national park is too small to ensure population survival in the long run. We recommend that protection be extended to the adjacent valleys, while allowing communities to sustain their livelihoods. These populations are also connected to the Indian population (Fig. 3), therefore protection of bears and habitat on the Indian side is equally important. Cross-border cooperation in this area should be a priority action for conservation of bears in the region, which may be a joint peace park or adjacent protected areas along the Line of Control. Such an initiative would benefit many other threatened large mammals as well, including the Asiatic black bear (*Ursus thibetanus*), common leopard (*Panthera pardus*), snow leopard (*Panthera uncia*), musk deer (*Moschus moschiferus*), and Himalayan ibex.

The average size of protected areas in South Asia is 400 km^2 (Ghazali and Khairi, 1995), and our study suggests that the DNP (with 1800 km^2 area) is not enough to ensure the long-term survival of a brown bear population. Therefore most of the existing PAs in South Asia may not be adequate to conserve populations of large mammals like brown bears.

The Himalayan brown bear is distributed throughout high Asia from Himalaya to Pamir and Tian Shan in small and often fragmented populations (Nawaz, 2007). Most of these populations are thought to be declining due to poaching and habitat loss (Roberts, 1997; Schaller, 1977, 1998; Servheen, 1990). Himalayan brown bears probably have a low reproductive rate throughout their range, because similar environmental conditions prevail all over high Asia. In the context of the low intrinsic growth potential, the conservation of these populations becomes more challenging. Nevertheless, our study documents that these low productive bears can be conserved. Population growth become much more sensitive to changes in survival rates when age of reproduction is delayed (Stearns, 1992). Documented mortalities in the park are predominantly human-caused; therefore the best strategy for conservation of brown bear in Himalaya, as elsewhere, is to reduce human-caused mortalities, particularly of females. Upholding a high level of survivorship in the population is a great effort, which requires the support of local communities to increase surveillance in the area and take timely action against poachers.

The presence of humans in occupied brown bear habitat is a reality, and the livelihood of local people is linked with it. Conservation planning based on the exclusion of people and implemented with force will therefore not succeed. The success of the DNP stresses the importance of integrating local people in planning and management of PAs. Changes to the legislative and regulatory framework of the PA that would recognize the rights of communities and provide the framework for community participation and benefit sharing would promote the involvement of the local people. Participation of local communities in the management process not only minimizes conflicts, but also leads to efficient conservation planning (Steinmetz et al., 2006).

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Table 1. Reproductive parameters of the brown bear population in Deosai National Park, Pakistan, 1993-2006. Calculated by method described in Garshelis et al. (1998).

Age of first reproduction

Age in years	Number of nulliparous females available to produce	Number producing young	% of nulliparous females producing	% of females in population available to produce	% in population producing	Age weighted by % of population producing
4	6	0	0	100	0	0
5	5	0	0	100	0	0
6	5	0	0	100	0	0
7	4	1	25.0	100	25.0	1.8
8	3	2	66.7	75.0	50.0	4.0
9	1	0	0.0	25.0	0.0	0.0
10	1	1	100.0	25.0	25.0	2.5
Sum		3			100	8.25
Mean						**8.25**
SD						3.99[a]

Litter interval

Years from one birth	Number of females with young	Number producing next litter	% of females producing next litter	% of females in population available to produce next litter	% in population producing next litter	Interval weighted by % of population producing next litter
1	24	0	0	100	0	0
2	21	0	0	100	0	0
3	17	0	0	100	0	0
4	14	3	21.4	100	21.4	0.9
5	9	3	33.3	78.6	26.2	1.3
6	5	2	40.0	52.4	21.0	1.3
7	3	2	66.7	31.4	21.0	1.5
8	1	1	100	10.5	10.5	0.8
Sum		11			100	5.7
Mean						**5.7**
SD						1.677[a]

Length of family association

Length of association (years)	Number of young associated with mothers	Number of young became independent	% of young becoming independent	% of available young in population	% in population becoming independe nt	Length weighted by % in population becoming independent
1.5	26	0	0	100	0.0	0.0
2.5	18	2	11.1	100	11.1	0.3
3.5	14	1	7.1	88.9	6.3	0.2
4.5	8	8	100	82.5	82.5	3.7
Sum		11			100	4.2
Mean						**4.2**
SD						3.063[a]

[a]SD calculated by bootstrapping

*One 9 year old nulliparous did not produce a litter by end of the study period, she was assumed to have cubs the next year (at age of 10 years) (Garshelis et al. 1998).

Table 2: Survival estimates and intrinsic population growth of brown bears in Deosai National Park, Pakistan, 1993-2006, using the Leslie matrix and Vortex methods. In the best-case scenario, undocumented losses were censored from the data ($\overline{S}_C$), whereas these individuals were treated as deaths ($\overline{S}_{AD}$) in the worst-case scenario.

	Best-case scenario	Worst-case scenario
	$\overline{S}_C$ (SD)	$\overline{S}_{AD}$ (SD)
Cubs-of-the-year	0.965 (0.034)	0.800 (0.067)
Yearling	1.00 (0.00)	0.848 (0.062)
$\geq$2 age group	0.976 (0.008)	0.923 (0.014)
λ estimates:		
Deterministic by Leslie matrix	1.032	0.964
Deterministic by Vortex	1.031	0.963
Stochastic by Vortex	1.030	0.965
95%CI on stochastic λ	0.968-1.093	0.794-1.135

Table 3. Comparison of reproductive parameters of the high-altitude brown bear population in Deosai, Pakistan, with other low-productive brown bear populations, and with the most productive populations yet documented, in Scandinavia.

Study area	AR[a]	LS[b]	LI[c]	RR[d]	Adult female weight Kg (n)	Cub survival	Reference (s)
Highly productive populations							
Central Sweden	5.2	2.3	2.4	0.96	117	0.65-0.83	Sæther et al. 1998, Swenson et al. 2001,
Northern Sweden	5.4	2.4	2.6	0.92	120	0.96	Sæther et al. 1998, Swenson et al. 2001,
High-latitude populations							
Anderson-Horton Rivers, NWT, Canada	6.0	2.27	4.90	0.78	-		Case and Buckland 1998
Kugluktuk NWT	8.7	2.26	3.30	0.87	-	0.81	Case and Buckland 1998
Nunavut-NWT	8.1	2.2	2.80	0.79[f]	-	0.74	McLoughlin et al. 2003
Tuktoyaktuk, NWT	6.4	2.3	3.30	0.7[f]	-		Ferguson and McLoughlin 2000; McLellan 1994
Northern Yukon	7.0	2.0	4.00	0.50[f]	116 (35)		Ferguson and McLoughlin 2000; McLellan 1994
West Brooks Range, Alaska	7.9	1.98	4.10	0.48[f]	117 (35)	0.56	Ferguson and McLoughlin 2000; McLellan 1994
Eastern Brooks Range, Alaska	9.6	1.78	4.20	0.42[f]	108 (31)		McLellan 1994
NW Alaska	6.1	-	3.90	-	-		Ferguson and McLoughlin 2000
High-altitude population							
Deosai National Park, Pakistan	8.25	1.33	5.7	0.23	73 (4)	0.94	This study

[a]Mean age of first reproduction (years), [b]Mean litter size, [c]Mean litter interval (years), [d]Reproductive rate/Natality (cubs/reproducing female/year), [f] RR calculated by LS/LI

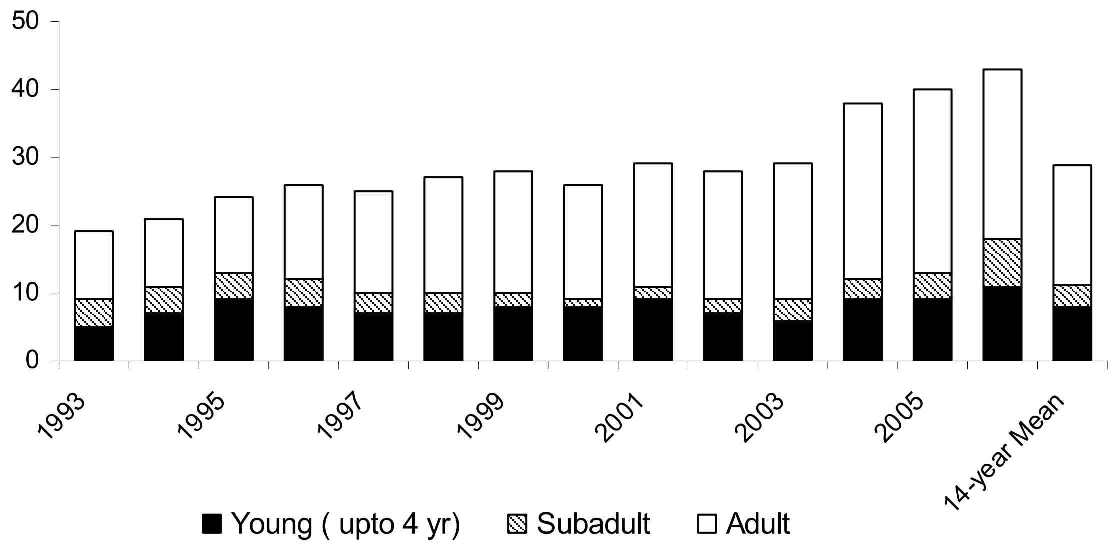
Figure legends:

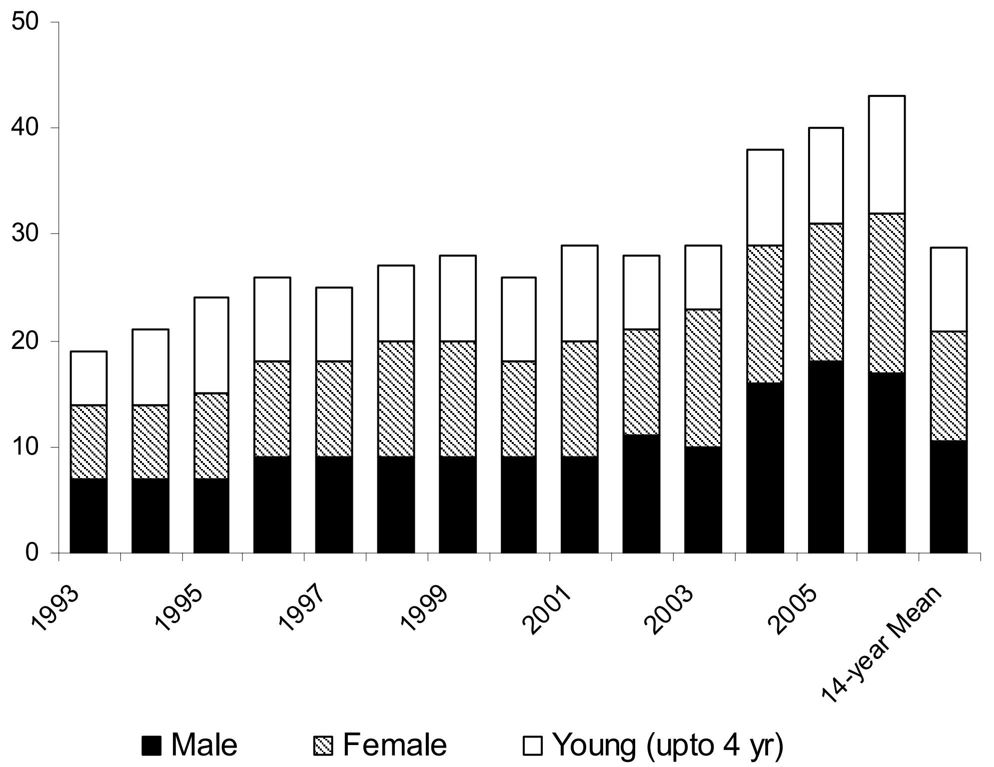
Figure 1. Age and sex structure of the brown bear population in Deosai National Park, Pakistan, from 1993 through 2006.

Figure 2. Growth of the brown bear population in Deosai National Park, Pakistan, from 1993 through 2006. Growth is shown on a natural log scale, the solid line shows the regression line, the inner dotted line is the 95% CI, and the outer broken line is the 95% prediction interval. Annual counts are shown as black circles.

Figure 3. Illustration of movements of brown bears between Deosai National Park and adjoining populations in the western Himalaya. (See Nawaz 2007 for spatial locations of these populations).

Figure 1.


50
40
30
20
10
0
1993
1995
1997
1999
2001
2003
2005
14-year Mean
Young (upto 4 yr)
Subadult
Adult


50
40
30
20
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0
1993
1995
1997
1999
2001
2003
2005
14-year Mean
Male
Female
Young (upto 4 yr)

Figure 2.

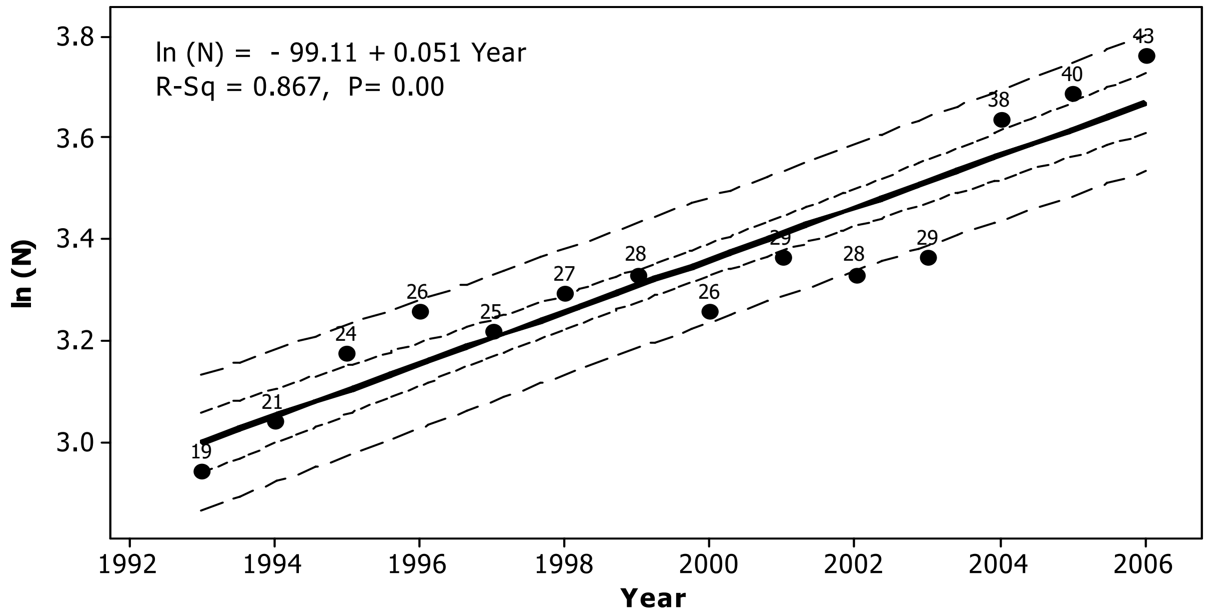
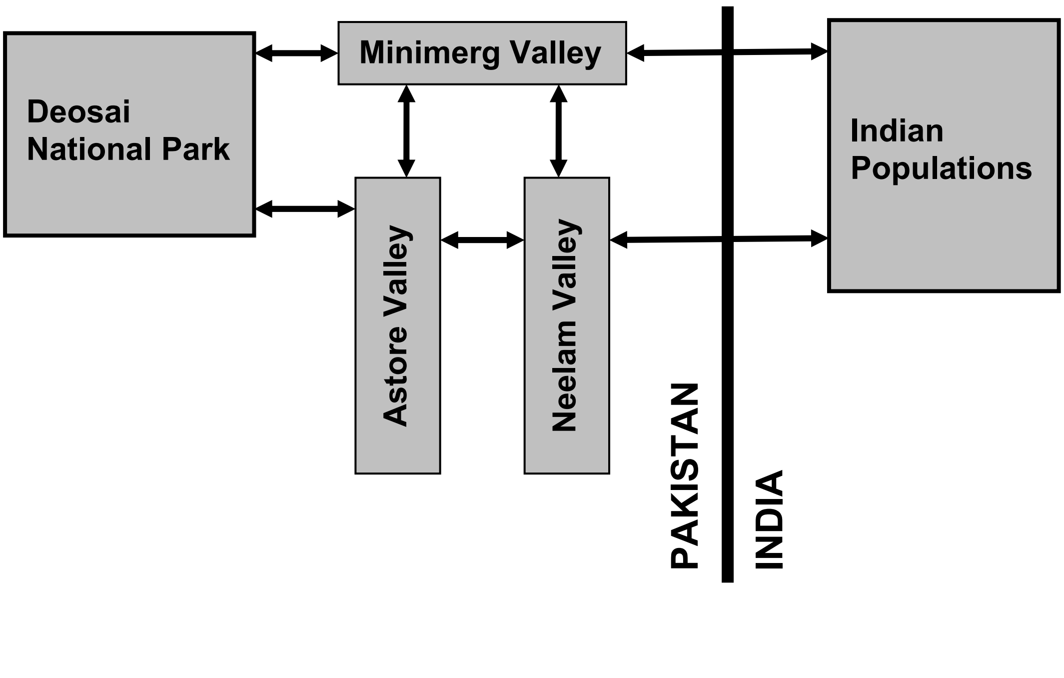


Figure 3.



Paper IV

NEW PERSPECTIVES IN DIET ANALYSIS BASED ON DNA BARCODING AND PARALLEL PYROSEQUENCING: THE *trn*L APPROACH

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Abstract

The development of DNA barcoding (species identification using a standardized DNA sequence), and the availability of recent DNA sequencing techniques offer new possibilities in diet analysis. DNA fragments shorter than 150 base pairs are usually degraded very slowly and can be recovered from faeces. As a consequence, by using universal primers that amplify a very short but informative DNA fragment, it is possible to reliably identify the plant taxon that has been eaten. According to our experience and using this identification system, about 50% of the taxa can be identified to species using the *trn*L approach, i.e. using the P6 loop of the chloroplast *trn*L (UAA) intron. We demonstrated that this new method is fast, simple to implement, and very robust. It can be applied for diet analyses of a wide range of phytophagous species at large scales. We also demonstrated that our approach is efficient for mammals, birds, insects, and molluscs. Undoubtedly, this method opens new perspectives in ecology, not only by allowing large-scale studies on diet, but also by enhancing studies on resource partitioning among competing species, and describing food webs in ecosystems.

Keywords: DNA barcoding, diet analysis, chloroplast DNA, faeces, *trn*L (UAA) intron, universal primers, pyrosequencing

Introduction

Trophic relationships are of prime importance for understanding ecosystem functioning (e.g. Duffy *et al.* 2007). They can only be properly assessed by integrating the diets of animal species present in the ecosystem. Furthermore, the precise knowledge of the diet of an endangered species might be of special interest for designing a sound conservation strategy (e.g. Marrero *et al.* 2004; Cristóbal-Azkarate & Arroyo-Rodrígez 2007).

Several methods have been developed to evaluate the composition of animal diets. The simplest approach is the direct observation of foraging behaviour. However, in many circumstances, direct observation is difficult or even impossible to carry out. It is often very time consuming or even impracticable when dealing with elusive or nocturnal animals, or when an herbivore feeds in a complex environment, with many plant species that are not separated spatially. The analysis of gut contents has also been widely used to assess the diet composition of wild herbivores foraging in complex environments (Norbury & Sanson 1992). Such an approach can be implemented either after slaughtering the animals, or by obtaining the stomach extrusa after anaesthesia.

Faeces analysis represents an alternative, non-invasive, and attractive approach. Up to now, four main faeces-based techniques have been used. First, for herbivores, microscope examination of plant cuticle fragments in faecal samples has been the most widely employed technique (Holechek *et al.* 1982; McInnis *et al.* 1983). This method is very tedious to perform, and requires a considerable amount of training and a variable proportion of plant fragments remains unidentifiable. Some herbivores do not masticate their food into small fragments, allowing plants present in the faeces to be identified visually (Dahle et al. 1998).

The second technique is based on the analysis of the natural alkanes of plant cuticular wax (Dove & Mayes 1996). This wax is a complex chemical mixture containing *n*-alkanes (saturated hydrocarbons) with chain lengths ranging from 21 to 35 carbons, and with the odd-numbered molecules largely predominating the even-numbered ones. There are marked differences in alkane composition among plant taxa (families, genera, species), and thus the alkane fingerprints represent a chemical approach for estimating the species composition. The approach is limited when the animal feed in complex environment. In this case it may be extremely difficult or impossible to have alkane concentrations in the samples that are representative of those present in the diet of the animal (Dove & Mayes 1996).

The third approach corresponds to Near Infrared Reflectance Spectroscopy (NIRS) (e.g. Foley *et al.* 1998; Kaneko & Lawler 2006). Near infrared spectra depend on the number and type of chemical bonds (C-H, N-H and O-H) present in the material being analyzed. After an appropriate calibration, the spectral features are used to predict the composition of new or unknown samples. The most common use of NIRS for diet analysis is the estimation of nutritional components in animal feeds, including total nitrogen, moisture, fibre, starch, etc. However this technique has several limitations. Particle size and particle homogeneity can bias the analysis. The calibration model is a crucial and challenging step, specific to the animal under study and to the species eaten.

The fourth method is based on DNA analysis by using either specific primers for a prey group or universal primers. The former procedure has been implemented by Deagle *et al.* (2007) for analyzing the diet of the Macaroni penguin (*Eudyptes chrysolophus*) using faeces as a source of DNA. The presence/absence of the different prey were detected by carrying out five different PCR assays using group-specific primers. Additionally, they also tested an approach involving universal 16S rDNA primers and subsequent cloning of the PCR products. These primers were designed to amplify DNA from fish, cephalopods and crustaceans, but to prevent the amplification of bird DNA. A good concordance was found between the diet deduced from DNA-based analyses of stomach contents and of faeces. Universal primers targeting the chloroplast *rbc*L gene and subsequent cloning have been used to analyze the diet of herbivorous species, either extinct species using coprolithes as a source of DNA (Poinar *et al.* 1998, 2001; Hofreiter *et al.* 2000, 2003), or living primates using fresh faeces (Bradley *et al.* 2007). The same type of DNA-based approaches was also performed for analyzing gut content in insects (see review in Symondson 2002) and in birds and mammals (e.g. Jarman *et al.* 2004).

In this paper we expand the DNA-based approach by combining the plant barcoding concept (Chase *et al.* 2005, 2007) with the new highly parallel sequencing systems (Margulies *et al.* 2005). More specifically, our goal is to describe a universal method for diet analysis of herbivorous animals by amplifying the P6 loop of the chloroplast *trnL* (UAA) intron (Taberlet *et al.* 2007) via the polymerase chain reaction (PCR; Mullis & Faloona 1987) and by subsequently sequencing individual molecules of this PCR product on the 454 automated sequencer (Roche Diagnostic, Basel, Switzerland). We demonstrate the efficiency of this new approach by analyzing the diet of various herbivorous species, including mammals, birds, molluscs, and insects.

Materials and methods

General strategy

Fig. 1 gives an overview of the main steps necessary to estimate the diet of herbivorous species. After collecting faeces in the field and extracting DNA, variable and short fragments of chloroplast DNA of the eaten plant species are amplified using universal primers. These fragments are subsequently sequenced. The plant taxa they come from are then identified using the DNA barcoding concept, by comparing the sequences obtained either with public databases (GenBank, EMBL, etc.) and/or with a database made for this purpose.

Faeces sampling

A total of 36 faeces samples were collected for analysis. For mammals, we sampled 12 faeces from golden marmots (*Marmota caudata*) in the Deosai National Park (Pakistan), with no more than one faeces per marmot colony. The marmot faeces were air-dried and preserved at room temperature in paper envelopes. We also analyzed 12 faeces from brown bears (*Ursus arctos*) collected in the same area, and previously used in another study for identifying individual bears (Bellemain *et al.* 2007). Brown bears are mainly vegetarian in this area, and the knowledge of its diet might have some conservation implications. Brown bear faeces were preserved in alcohol. For birds, we used six capercaillie (*Tetrao urogallus*) samples previously analysed in Duriez *et al.* (2007), four from the French Pyrenees (*T. u. aquitanus*) and two from the Corinthian Alps in Austria (*T. u. major*). Capercaillie faeces were preserved dry in silica gel. For the invertebrates, we collected three grasshopper faeces (two from *Chorthippus biguttulus*, and one from *Gomphocerippus rufus*) and three mollusc faeces (from the snail *Helix aspersa*, and from the slugs *Deroceras reticulatum* and *Arion ater*). Insect and mollusc faeces were also preserved dry in silica gel.

DNA extraction from faeces

Total DNA was extracted from about 10 mg of sample with the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions, except for the three grasshopper samples where the whole faeces were used. The DNA extracts were recovered in a total volume of 300 µL. Mock extractions without samples were systematically performed to monitor possible contaminations.

DNA amplification

DNA amplifications were carried out in a final volume of 25 µl, using 2.5 µl of DNA extract as 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). After 10 min at 95°C (*Taq* activation), the PCR cycles were as follows: 35 cycles of 30 s at 95°C, 30 s at 55°C; the elongation was removed in order to reduce the +A artefact (Brownstein *et al.* 1996; Magnuson *et al.* 1996). Each sample was amplified with primers *g* and *h* (Taberlet *et al.* 2007), 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.

DNA sequencing

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 faeces samples.

Large-scale pyrosequencing was carried out on the 454 sequencing system (Roche, Basel, Switzerland) following manufacturer's instructions, and using the GS 20 for marmot and bear, and the GS FLX for other samples.

DNA barcoding database for the Deosai National Park

In order to more precisely assess the diets of brown bears and golden marmots in Deosai National Park, leaves of the most common plant species occurring in this alpine environment were collected and identified by three botanists (Dr Muhammad Qaiser, Dr Muqarrab Shah, and Dr. Mir Ajab

Khan). The database was elaborated by sequencing the whole chloroplast *trn*L (UAA) intron of these species using the *c* - *d* primer pair (Taberlet *et al.* 1991), and following the protocol described in Taberlet *et al.* (2007).

Data analysis for estimating diet composition

Out of the mix of sequences obtained after the pyrosequencing, the first step of 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 faeces), 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. The diet was then determined by comparing these sequences to the homologous sequences available in databases. In the case of the brown bear and marmot, the sequences were first compared to the database generated for the Deosai National Park and then, if no match was found, to public databases. For all other species, the sequences were directly compared to public databases to find their closest match using the MEGABLAST algorithm (Zhang *et al.* 2000).

Results

DNA barcoding database for the Deosai National Park

The chloroplast *trn*L (UAA) intron was sequenced for 91 plant species belonging to 69 genera and 32 families. Seventy-five percent of the species analyzed have a unique P6 loop sequence (i.e. the sequence amplified with the *g* - *h* primer pair) and thus can be identified to species. Of the remaining 25 %, 20 % could be identified to genus, and 5 % to family. All these sequences have been deposited in EMBL database, under accession numbers EU326032-EU326103.

Pyrosequencing results

For the analysis of the 36 faeces, we obtained a total of 97,737 P6 loop sequences, corresponding to an average of 2715 ± 1130 sequences per sample. In each samples, a few sequences were found hundreds of time, whereas some other sequences are only represented either once or by very few occurrences (Table 1). The sequences showing up only once, twice, or three times were not taken into account in the subsequent analysis. They were almost always very close to a highly represented sequence, and thus considered to be the result of sequencing errors in the P6 loop. In rare cases, we also found sequences represented only once, that were not close to a highly represented sequence. Such sequences most likely correspond to a sequencing error within the tag, leading to an assignment to a wrong sample. This observation led us to modify our tagging system (see Discussion).

DNA-based diet analysis

The DNA-based diet analyses of marmots and bears are summarized in Table 2 and Fig. 2. Sixty-four percent and 31% of the different P6 loop sequences obtained in their diet was identified to species for marmots and bears, respectively. Overall, the marmot has a much more eclectic diet, with 28 species identified (out of the 779 different P6 loop sequences), belonging to 15 families. Only 557 different P6 loop sequences were identified in the brown bear diet, which is composed mainly of Poaceae and Polygonaceae, with a significant contribution of Cyperaceae and Apiaceae.

Table 3 gives the results obtained for the birds, molluscs, and insects. All these results are consistent with what we know about the diet of these animals, particularly for capercaillie, which eat mainly conifers in winter, and grasshoppers, which eat mainly grasses.

Discussion

Using faeces as a source of DNA, and by combining universal primers that amplify a very short but informative fragment of chloroplast DNA and large-scale pyrosequencing, we were able to successfully assess the diet composition of several herbivorous species. This DNA-based method is broadly applicable to potentially all herbivorous species eating angiosperms and gymnosperms, including mammals, insects, birds, and molluscs.

Such an approach has many advantages over previous methods used for diet analysis (i.e. microscope examination of plant cuticle fragments, chemical analysis of alkanes, NIRS). Our approach is robust and reliable, in relation to the very short length of the amplified region. The primers target highly conserved regions in angiosperms and gymnosperms, preventing strong bias in the efficiency of amplifications among species. The two highly conserved regions targeted by these primers flank a short and variable region that allows the identification of the plant taxa. The results obtained in marmots show clearly that the system is particularly well adapted for analyzing complex situations, when the diet is composed of many different species. This approach can be coupled with individual identification using microsatellite polymorphism (Taberlet & Luikart 1999), allowing diet comparisons among individuals, even without observing the animals. An alternative and very inexpensive approach could involve the pooling of many faeces in the same DNA extraction in order to obtain the average diet composition directly, but this strategy would prevent the analysis of individual diets.

The *trn*L approach represents a significant progress in plant identification when using faecal material. The same standardized method is easy to implement and can be applied to a wide range of animal species. It is particularly well suited for large-scale analyses, with the possibility to analyze several hundreds of samples in the same 454 GS FLX sequencing run and to automate the sequence analysis by implementing bioinformatic tools. This offers the prospect of following the diet composition over seasons and of comparing among age classes, individuals, and sexes. Within the same species, it also allows the analysis of diet shifts according to plant availability and food preferences.

However, this method still has some limitations, and it is clear that the resolution does not reach the species level in all cases. However, by building a comprehensive database of *trn*L (UAA) introns for the majority of the plant species that occur in a particular area, usually about 50% of the different species should be identified to species, and 90% to genus. It is interesting to note that some genera exhibit a limited variation (e.g. *Carex*) or almost no variation (e.g. *Salix*, *Pinus*, etc.) on this P6 loop. When it is important to determine the species, we suggest to complement the universal *trn*L approach by one or several additional systems, specially designed for amplifying a short and variable region in these genera. According to the availability of more and more DNA sequences in databases, primer pairs can be designed that are specific to these problematic genera. These primers might target other more variable parts of the chloroplast DNA, or the nuclear ribosomal DNA, such as the internal transcribed spacers.

We would like to highlight two potential difficulties of our approach, linked to the sequencing strategy using a huge mix of DNA molecules, and to the sequencing errors observed with the 454 sequencer. The 454 sequencer produces several hundreds of thousands of sequences per run, in a single file containing unsorted sequences corresponding to the mix of DNA molecules. The only way to reduce costs, while still producing many sequences per sample, is to pool many PCR products before the sequencing step. As a consequence, we tagged each sample differently in order to find the corresponding sequences in the sequencer output. Our first tagging system added a 5'-CCNNNN-3' tag to the 5' end of the primers. However, due to the occurrence of sequencing errors within the tags, either substitutions or indels (insertions/deletions), we suggest to improve the tagging system by using the following sequence: 5'-CCDNNNN-3' (D = A or G or T), with at least two differences among tags and avoiding stretches of the same nucleotide longer than two (Gielly *et al.* in preparation). The second difficulty comes from the sequencing errors within the P6 loop itself. Such errors can come from the degradation of the template DNA in faeces, from nucleotide misincorporation during DNA amplification, or from the sequencing process itself. The 454 sequencer is known for having difficulty in counting the exact number of repeats of the same nucleotide, even in short stretches of three or four nucleotides. We also observed many substitutions, and indels not linked to stretches (see Table 1). All these errors make the species identification more complex. Nevertheless, the exact sequences are usually present in a high copy number, whereas those containing errors occur at a low frequency (see Table 1). In this first study, we only considered sequences present at least four times. It is clear that the method can be improved significantly by a better knowledge of the type of the different sequencing errors and of their associated probabilities. The availability of a *trn*L (UAA) intron database with the plant species available in the study area greatly facilitates plant identification when using the *trn*L approach for diet analyses.

Another potential difficulty is the risk of contamination, from the sampling step in the field to the sequencing step. The *g* - *h* primer pair is highly efficient, and we do not recommend carrying out more than 35 amplification cycles, except if strong measures are taken to avoid potential contaminations, as in ancient DNA studies. During a pilot experiment, we noticed that samples extracted with the Qiagen Stool Kit (Qiagen GmbH, Hilden, Germany) systematically contained potato DNA, most likely coming from the "inhibitex" pill used during the extraction process. Qiagen technical support confirmed that "it cannot be ruled out that Inhibitex may contain DNA from plants". As a consequence, we recommend to avoid the Qiagen Stool Kit when amplifying plant DNA.

An important aspect in diet analysis is the absolute or relative quantification of the different plant species that have been eaten. The *trn*L approach provides the number of molecules after DNA amplification. However, these numbers cannot be interpreted as quantitative at the moment for several reasons. First, the preferential amplification of some species when analyzing a mixture of templates is well known (Polz & Cavanaugh 1998). The fact that the *g* - *h* primer pair targets highly conserved regions, with almost no variation (Taberlet *et al.* 2007), should limit such preferential amplification. Additionally, new technologies, such as emulsion PCR, can minimize this problem and at the same time should enable the quantification of DNA fragments in a mix (Williams *et al.* 2006). Second, the amount of template DNA (chloroplast DNA) clearly varies among the type of tissue eaten. Leaves will undoubtedly provide more chloroplast DNA than roots, and the *trn*L approach cannot determine the tissue that has been eaten. Knowing the species eaten, the NIRS method has the potential of providing information about the tissue eaten. Third, the *trn*L approach alone cannot assess the absolute quantity of the different plant species eaten. Thus, it provides an estimate of the frequency of occurrence of a food item in the faeces, but not an estimate of the volume eaten. In simple conditions, i.e. when the animal is eating only a few species and is additionally feed with a known amount of even-numbered alkane molecules, the alkane approach can supply estimates of the absolute quantity of plant eaten (Dove & Mayes 1996). Consequently, the *trn*L, the NIRS, and the alkane approaches should be considered as complementary.

Non-invasive genetic studies are very attractive and now extensively used, especially when dealing with endangered species. With this new *trn*L approach for diet analysis, we widen the field of non-invasive analysis using faeces as a source of information. This opens new perspectives in conservation biology and more generally in ecological studies by enhancing research on resource partitioning among competing species, and describing food webs in ecosystems.

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Figure legends

Fig. 1 Flowchart diagram showing the main steps of the *trn*L approach for assessing diet composition using faeces.

Fig. 2 Comparison of the diet compositions of the golden marmot (*Marmota caudata*) and of the brown bear (*Ursus arctos*) in the Deosai National Park (Pakistan). See Table 2 for the plant taxa identified within each of these families. The Y-axis corresponds to the frequency of presence of taxa from the same family in the twelve samples of each mammal species.

Tables and Figures

Table 1 P6 loop (chloroplast *trn*L (UAA) intron) sequences obtained after high throughput pyrosequencing for the bird faeces sample n° 5 (*Tetrao urogallus major*). A total of 3546 sequences were obtained with an occurrence higher than three. The diet was composed of two plant taxa: *Picea* and *Abies*. Besides the most common sequences for each of these two taxa, it is interesting to note the presence of sequence variants due to errors originating from the degradation of the template DNA in faeces, from nucleotide misincorporation during DNA amplification, or from the sequencing process on the 454 sequencer.

Number of occurrences	P6 loop (chloroplast *trn*L (UAA) intron) sequences	Identification
3103	`ATCCGGTTCATGGAGAC-AATAGTTT-CTT-CTTTTATTCTCCTAAGATA-GGAAGGG`	*Picea*
45	`.................-........-...-....-..............-.......`	*Picea* variant
42	`.................-........-...-...................-......-`	*Picea* variant
13	`..........................-...-...................-......A`	*Picea* variant
9	`.................-........-...T...................-.......`	*Picea* variant
9	`.................-........-...-.......C...........-.......`	*Picea* variant
6	`.................-........-..C-...................-.......`	*Picea* variant
6	`.................-........-...-...C...............-.......`	*Picea* variant
6	`.................-........-...C....-..............-.......`	*Picea* variant
5	`............A....-........-...-...................-.......`	*Picea* variant
5	`.................-........-...-........T..........-.......`	*Picea* variant
5	`.................T........-...-...................-.......`	*Picea* variant
5	`.................-.G......-...-...................-.......`	*Picea* variant
5	`.................-........-...-...................A.......`	*Picea* variant
5	`.................-........T...-...................-.......`	*Picea* variant
5	`.................A.T......-...-...................-.......`	*Picea* variant
4	`.................-........-...-...................-....A..`	*Picea* variant
4	`-................-........-...-...................-.......`	*Picea* variant
4	`................T-........-...-...................-.......`	*Picea* variant
4	`.................-........-...-...................G.......`	*Picea* variant
4	`......C..........-........-...-...................-.......`	*Picea* variant
4	`.................-........-...-....-..............-......-`	*Picea* variant
4	`.................-........-...-...................-...G...`	*Picea* variant
4	`..............A..-........-...-...................-.......`	*Picea* variant
236	`ATCCGGTTCATAGAGAAAAGGGTTTCTCTCCTTCTCCTAAGGAAAGG`	*Abies*
4	`..................-............................`	*Abies* variant

Table 2 Plant taxa identified in the diet of the Himalayan brown bear (*Ursus arctos*) and of the golden marmot (*Marmota caudata*) in Deosai National Park (Pakistan), based on sequence variation of the P6 loop of the chloroplast *trn*L (UAA) intron using faeces as a source of DNA.

			Ursus arctos													***Marmota caudata***												
			Faeces sample													Faeces sample												
Family	**Plant taxon**	**Level of identification**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Apiaceae	Apoideae	subfamily				x									1													-
	Heracleum candicans	species		x		x					x	x		x	5		x					x		x				3
	Pleurospermum hookeri	species													-				x	x	x			x				4
Araceae	Araceae*	family													-			x										1
Asteraceae	*Anaphalis nepalensis*	species													-										x			1
	Anthemideae_1*	tribe				x									1		x		x	x		x	x	x	x	x		8
	Anthemideae_2*	tribe													-				x	x		x			x			4
	Aster falconeri	species													-				x			x	x		x		x	5
	Asteraceae_1*	family													-				x									1
	Asteraceae_2*	family		x										x	2				x	x	x	x			x	x		6
	Asteraceae_3*	family													-			x	x									2
	Asteraceae_4*	family													-						x				x			2
	Asteraceae_5*	family													-						x				x			2
	Asteraceae_6*	family													-											x		1
	Asteroideae_1*	subfamily													-		x	x		x	x	x	x	x		x		8
	Asteroideae_2*	subfamily													-					x		x			x	x		4
	Asteroideae_3*	subfamily													-					x								1
	Asteroideae_4*	subfamily													-				x									1
	Coreopsideae*	tribe													-			x		x	x							3
	Gnaphalieae*	tribe													-					x								1
	Inuleae*	tribe									x				1			x		x			x			x		4
	Leontopodium brachyactis	species													-							x						1
Brassicaceae	Brassicaceae	family													-										x			1
	Draba oreades	species													-			x			x							2
	Thlaspi andersonii	species													-	x					x							2
Cannabaceae	*Cannabis sativa**	species													-									x				1
Caryophyllaceae	*Cerastium*	genus					x								1	x		x	x		x	x	x		x	x	x	9
	Cerastium cerastoides	species										x		x	2	x		x	x		x	x	x	x	x	x	x	10
	Cerastium pusillum	species					x								1	x		x							x	x	x	5

			Ursus arctos													*Marmota caudata*												
			Faeces sample													Faeces sample												
Family	**Plant taxon**	**Level of identification**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
	*Silene**	genus													-	x		x										2
	Silene tenuis	species													-	x								x		x		3
Crassulaceae	Crassulaceae	family													-				x	x		x		x				4
	Rhodiola	genus													-		x											1
Cyperaceae	*Carex*	genus		x		x	x		x		x	x		x	7													-
	Carex diluta	species		x		x	x				x	x		x	6													-
Fabaceae	*Astragalus rhizanthus*	species		x											1	x	x	x	x	x		x	x	x	x			9
	Galegeae	tribe		x											1	x			x			x						3
	Oxytropis cachemiriana	species													-	x		x	x		x			x		x	x	7
Juncaceae	*Juncus**	genus									x				1													-
Lamiaceae	*Dracocephalum nutans*	species													-		x	x										2
	Mentheae	tribe			x	x									2	x	x	x	x	x			x	x		x		8
Onagraceae	*Chamerion latifolium*	species													-			x										1
Orobanchaceae	*Pedicularis*	genus		x											1													-
	Pedicularis albida	species		x											1													-
Papaveraceae	*Papaver nudicaule*	species													-	x	x											2
Pinaceae	*Cedrus**	genus			x										1													-
	*Picea**	genus													-			x										1
Plantaginaceae	*Lagotis kunawurensis*	species													-											x		1
	*Plantago**	genus													-											x		1
Poaceae	*Agrostis vinealis*	species	x			x		x	x	x		x			6								x					1
	Elymus longi-aristatus	species													-						x		x		x			3
	Poa alpina	species													-						x							1
	Poa	genus												x	1													-
	Poa supina	species													-					x	x			x			x	4
	*Pooideae**	subfamily	x	x	x	x	x	x	x	x	x	x	x	x	12	x		x			x	x	x	x		x		7
Polygonaceae	*Aconogonon rumicifolium*	species			x							x	x		3		x		x	x								3
	Bistorta affinis	species				x		x				x		x	4													-
	Polygonaceae	family													-							x		x	x			3
	Polygonum cognatum	species													-	x		x			x							3
	*Rumex**	genus									x				1	x		x	x	x	x	x		x	x			8

			Ursus arctos													***Marmota caudata***												
			Faeces sample													Faeces sample												
Family	**Plant taxon**	**Level of identification**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
	Rumex nepalensis	species									x				1	x		x	x	x	x	x			x			7
Ranunculaceae	*Aconitum violaceum*	species										x			1													-
Rosaceae	*Cotoneaster affinis*	species													-											x		1
	Potentilla argyrophylla	species													-	x	x	x			x			x				5
	Rosoideae	subfamily												x	1	x	x			x		x		x				5
Rubiaceae	*Galium boreale*	species												x	1		x											1
Saxifragaceae	*Saxifraga hirculus*	species											x		1												x	1
Solanacee	*Solanum**	genus													-						x	x						2
Total number of plant species per faeces			2	9	4	9	5	3	3	2	8	9	3	10		17	12	21	18	18	20	19	11	17	17	16	7	

* Plants identified by comparing the sequence with sequence data in public databases.

Table 3 Plant taxa identified in the diet of birds, molluscs, and insects based on sequence variation of the P6 loop of the chloroplast *trn*L (UAA) intron using faeces as a source of DNA.

Family	Plant taxon	Level of identification	B1	B2	B3	B4	B5	B6	M1	M2	M3	I1	I2	I3
Apoideae	Apoideae	family											x	
Asteraceae	Asteraceae	family								x	x			
Brassicaceae	Brassicaceae	family							x					
Ericaceae	Rhodoreae	tribe	x											
Fagaceae	Fagaceae	family						x						
Lamiaceae	Nepetoideae	subfamily									x			
Linnaeaceae	Linnaeaceae	family	x											
Oleaceae	Oleaceae	family								x				
Pinaceae	*Abies*	genus					x							
	Picea	genus					x	x						
	Pinaceae	family						x						
	Pinus	genus	x	x	x	x		x						
Plantaginaceae	*Veronica*	genus									x			
	Veroniceae	tribe								x				
Poaceae	*Bromus*	genus											x	x
	Holcus lanatus	species												x
	Hordeum	genus											x	
	Poae	tribe											x	
	Pooideae	subfamily										x	x	x
Ranunculaceae	Ranunculus	genus				x								
Rosaceae	Maloideae	subfamily								x	x			
	Prunus	genus									x			
Total number of plants per faeces			3	1	1	2	2	4	1	4	5	1	5	3

B1 = *Tetrao urogallus aquitanus* Sample 1; B2 = *T. u. aquitanus* Sample 2; B3 = *T. u. aquitanus* Sample 3; B4 = *T. u. aquitanus* Sample 4; B5 = *T. u. major* Sample 1; B6 = *T. u. major* Sample 2; M1 = *Helix aspera*; M2 = *Deroceras reticulatum*; M3 = *Arion rufus*; I1 = *Chorthippus biguttulus* Sample 1 (male); I2 = *C. biguttulus* Sample 2 (female); I3 = *Gonfophocerippus rufus*.

Figure 1

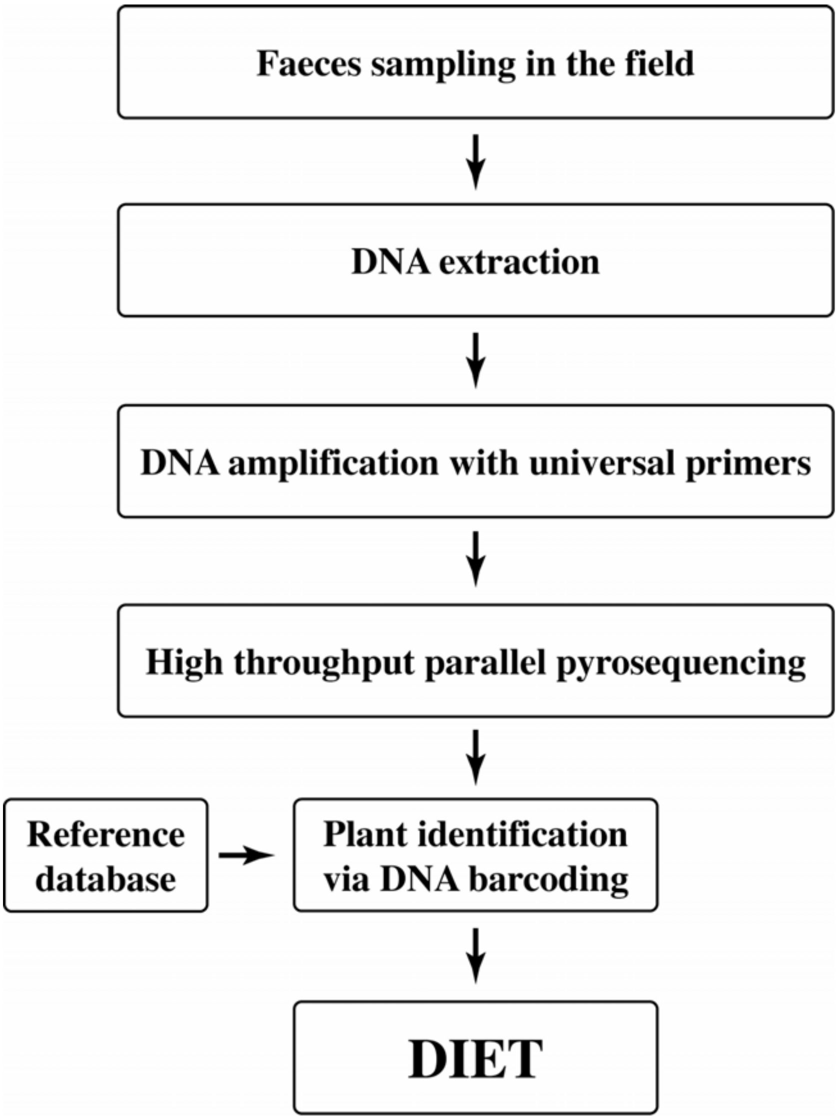
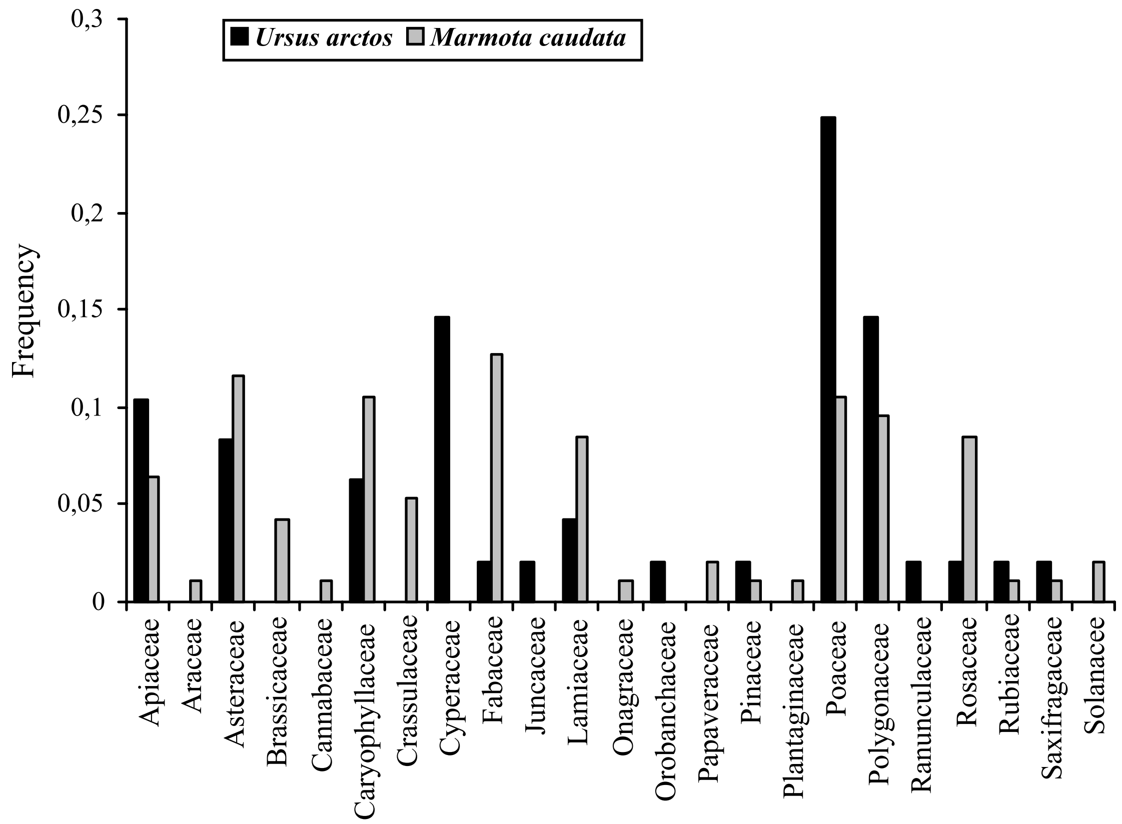

Faeces sampling in the field
DNA extraction
DNA amplification with universal primers
High throughput parallel pyrosequencing
Reference database
Plant identification via DNA barcoding
DIET

Figure 2



Ursus arctos
Marmota caudata
Frequency
0,3
0,25
0,2
0,15
0,1
0,05
0
Apiaceae
Araceae
Asteraceae
Brassicaceae
Cannabaceae
Caryophyllaceae
Crassulaceae
Cyperaceae
Fabaceae
Juncaceae
Lamiaceae
Onagraceae
Orobanchaceae
Papaveraceae
Pinaceae
Plantaginaceae
Poaceae
Polygonaceae
Ranunculaceae
Rosaceae
Rubiaceae
Saxifragaceae
Solanacee

Paper V

DIET OF THE BROWN BEAR IN HIMALAYA: COMBINING CLASSICAL AND MOLECULAR GENETIC TECHNIQUES

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ABSTRACT

The ecological requirements of brown bears are poorly known in Himalaya, which complicates conservation efforts. We documented the diet of the Himalayan brown bear 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. However, graminoids made up 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 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 food.

The estimated digestible energy available to brown bears in Deosai National Park was the lowest documented in brown bear populations, due to the lack of fruits and a relatively lower meat content in the diet. The low nutritious diet and high cost of metabolism in a high altitude environment, probably explains the very low reproductive potential of this population.
Keywords: brown bear, diet, energy, high altitude, Himalaya, mammal, Pakistan, reproduction

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 of species from an ecological perspective (Robbins 1993; Sih 1993). These 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, understanding of nutritional ecology of bears has advanced significantly in the past two decades (Robbins and Schwartz 2004). However most diet studies of brown bear have been conducted in North America (e.g; Hamer and Herrero 1987; McLellan and Hovey 1995; Mealey 1980) or in Europe (e.g; Clevenger et al. 1992; Dahle et al. 1998). The brown bear (*Ursus arctos*) is an opportunistic omnivore with a wide geographical distribution (Schwartz et al. 2003) and utilizes food according to local availability (Craighead et al. 1995; LeFranc et al. 1987). Therefore the knowledge of diet from North America or Europe can not be generalized for other geographical locations. There is limited information on the diet of brown bears in Asia (Nomura and Higashi 2000; Ohdachi 1987; Xu et al. 2006), particularly no studies exist from the Himalaya, Karakoram and Hindu Kush ranges in South Asia. In order to plan and implement an effective conservation programs for brown bears in Himalaya, a sound knowledge of nutritional ecology is essential (Robbins and Schwartz 2004).

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 (Nawaz 2007). 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 (Bunnell and Tait 1981). 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) (Paper III). This study showed that the brown bear population in DNP is the least productive in the world. A positive relationship between diet of bears and their reproductive performance has been documented in a wide range of studies (Hilderbrand et al. 1999; Jonkel and Cowan 1971; Rogers 1987; Schwartz and Franzmann 1991; Stringham 1990). In North America, >90% of the variation in age of first reproduction was explained by vegetational productivity (Ferguson and McLoughlin 2000). Autumn body mass, which is dependant on local food conditions, is an important indicator of reproductive output in bears (Rogers 1987; Schwartz and Franzmann 1991; Stringham 1990).

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 else where.

We also investigated temporal and habitat effects, because seasonal and habitat variation in diet has been reported for brown bears (Craighead et al. 1995; Dahle et al. 1998; MacHutchon and Wellwood 2003; McLellan and Hovey 1995; Welch et al. 1997). Mattson (2000) suggested that gender-related nutritional needs may result in sex differences in diet. Though not consistent in all studies (Case and Buckland 1998; Powell and Zimmermann 1997), male bears often eat more meat than females (Boertje et al. 1988; Felicetti et al. 2005;

Hobson et al. 2000; Jacoby et al. 1999; Mattson 1997). We tested if sex-related differences exist in the selection of plant species or in overall diet items.

MATERIALS AND METHODS

Study area.— DNP, about 1800 km^2, occupies part 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 (Paper VI). 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 (Paper VI). 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 (October-May, depending on weather). 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 from the park, but some (43) were collected from 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 Bellemain et al.

2007). 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 (Nawaz et al. 2006). 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, Taberlet et al. 2007), and 73 with identification at the species level were added to GenBank (accession numbers EU326032-EU326103, Nawaz 2008). 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 (Woods et al. 1997), and were relatively abundant (pers. obs.). The ground-dwelling invertebrate fauna in DNP was sampled in 1999 (Kok et al. 2005). 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*) (Khan 1962) 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. (1997) recorded seven small mammal species in DNP (*Alticola argentatus, Sicista concolar, Sorex thibetanus, Hyperacrius fertilis, Marmota caudata* (golden marmot)*, Mustelia erminea, Ochotona roylei*) and provided their relative numbers. *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 (Blumstein and Arnold 1998) 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 (Nawaz and Kok 2004), we expected that marmots would be an important component of brown bear diet. Thus our study focused on estimating 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, Blumstein and Arnold 1998) 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, Paper IV) 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 et al. 1998; Elgmork and Kaasa 1992). 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 (Mattson et al. 1991). 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 (Bellemain et al. 2007). 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 (Brownstein et al. 1996; Magnuson et al. 1996). Each sample was amplified with primers *g* and *h* (Taberlet et al. 2007), 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 (Zhang et al. 2000).

We plotted the frequencies of identified families, and classified them as regular (≥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. (2007) 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. 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 (Hewitt and Robbins 1996; Mealey 1980) and nutritional composition (Pritchard and Robbins 1990), 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 (Hewitt and Robbins 1996) to RV. We used the following CFs: 4 for rodents, 3 for ungulates and 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 Pritchard and Robbins (1990); ungulates = 29.4 kj/g, rodents = 22.1, and invertebrates = 17.7 (Johansen 1997). The digestible energy (kj/g) for plants in DNP was estimated as; graminoids:11.8, forbs: 11.2, and shrubs: 12.2 (Nawaz 2008).

Sex variation. — Bellemain et al. (2007) 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 (Agresti 1996). 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 (Agresti 1996). 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 (1996):

Logit (π) = $\alpha + \beta_i^X$; X= factor of month with levels i =1,2,3,4 (June-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. — 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, respectively) but highest in marshy habitat (26 colonies per km^2). The three habitats supporting marmots cover about 65% of the park (Paper VI). 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). Seventy percent scats 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). The diet category with the second highest frequency was forbs, at 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 rodents 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 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 of Cyperaceae (*Carex diluta, Carex* sp.), and one 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.

Sex differences in diet. — Scat analysis of 43 samples, for which sex was known (Fig. 2), indicated that the behavior of the sexes with respect of individual food item 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.

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 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 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 are 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 still 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 volume of forbs (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 omnivore species like brown bear. The *trn*L approach provided a more accurate descrption of plant diversity in the diet and its frequency. The scat analysis helped ascertaining 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 (Krebs and McCleery 1984; Manley et al. 2002). However, in 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 (McLellan and Hovey 1995). Looking at plant and animal resources separately, we found consumption in accordance with availability. Graminoids comprise the highest biomass in park, followed by forbs (Paper VI). Shrubs, which are restricted to thin 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 the 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 the Deosai National Park was established in 1993, there was no resident population of ungulates (HWF 1999). 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 bears in the park. Domestic livestock were also 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 (Kok et al. 2005). 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 (Swenson et al. 1999) or moth aggregation sites in North America (White et al. 1999), where they make a significant contribution to energy assimilation in 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 scats, which is a limitation of scat analysis reported earlier (Cicnjak et al. 1987). However underestimation of the volume of forbs may not have been greater, 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 distribution of main food plants (Clark et al. 1994; Costello and Sage 1994), though those plants might be eaten due to their greater availability rather than selective preference (Nomura and Higashi 2000). We documented that brown bears prefer marshy habitats in DNP (Paper VI). 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 (Paper VI). Secondly, during a time budget study, bears were mostly observed in marshy habitats where their dominant activity was grazing (Nawaz and Kok 2004). Thirdly, the highest density of brown bears occurs in the Black Hole area (central part of the park), which is predominantly a marshy habitat (Paper III). Thus the graminoids are the most abundant and concentrated source of food for bears, and key factor explaining resource selection by brown bears. In agreement with our results, brown bears using alpine habitat in Alaska are heavily dependant on graminoids (Atwell et al. 1980).

Vertebrates that depend on plant matter for their nutritional requirements exhibit digestive track modifications, either through compartmentalization of for-gut or an elaborate sacculation of hind-gut (Stevens and Hume 1998). These specializations aid in retention of digesta and harbor microbial populations that convert indigestible plant matter (cell wall components) into absorbable nutrients (Soest 1994; Stevens and Hume 1998). The brown bear possess an anatomically simpler gastrointentinal tract like other carnivores (Davis 1964; Stevens and Hume 1998). Although two adaptations, an extremely large intestine and bunodont molars, make the brown bear a more efficient digester of plant matter 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 (Clemens and Stevens 1979; Soest 1994). 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* (Dierenfeld et al. 1982; Soest 1994). The retention time of plant food in brown bears is also very short (7±0.8 hour, Pritchard and Robbins 1990), however the relatively larger intestine may increase absorption (Stevens and Hume 1998). 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, Faichney and White 1988; Stevens and Hume 1998; Udén et al. 1982). Although the digestion of structural carbohydrates is insignificant in brown bears due to fast passage (Mealey 1980), the loss of cell soluble is however small (protein digestion is only 5% lower than in ruminants, Pritchard and Robbins 1989). The high intake rate of brown bears is supported by the time budget study in DNP (Nawaz and Kok 2004), where bears were observed spending largest part of the day foraging (67% of day light 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 (Schwartz et al. 2003), males are about 50 % heavier than females in DNP (Paper III). Larger body size increases the reproductive success of males through; 1) increasing chances of fertilization in a promiscuous mating system (Craighead et al. 1995; Schenk and Kovacs 1995) because ejaculate volume is correlated with size (Erickson et al. 1968), and 2) increasing social dominance, which increases access to reproductive females (Craighead et al. 1995). 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 (Hilderbrand et al. 1999). Golden marmots made up the major meat source in DNP, and capturing requires a lot of 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 (Craighead et al. 1995; Hamilton and Bunnell 1987; McLellan and Hovey 1995). For example in central Sweden; ungulates are the main diet in spring, whereas ants, forbs, and ungulates dominate in summer, and berries dominate the autumn diet (Dahle et al. 1998). In DNP, we did not find a significant temporal impact, 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 (Graham 1978; Hamer and Herrero 1987). During the late growing season, before denning, bears show hyperphagy (Nelson et al. 1983) and may increase their intake of high nutritious food (meat) if available (McLellan and Hovey 1995). 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 (Blumstein and Arnold 1998), which prevents body temperatures falling below a critical threshold through coordinated bouts of social thermoregulation (Arnold 1993; Arnold et al. 1991). Blumstein and Arnold (1998) 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. Though brown bears foraged until October in DNP, limited activity by marmots probably explains the lack of increase in meat intake in later months.

Anthropogenic foods are found in brown bear food when bears coexist with humans (Schwartz et al. 2003). Human-related food in the present study was predominantly crops, and in a few scats we found cultivated fruits (citrus, kiwi) and garbage (food packing). 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 (Paper III). 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 protein content was low compared with other brown bear populations with comparable data (Table 4). However Westerterp and Kayser (2006) suggested that carbohydrates are a preferable energy source as compared with proteins at high altitudes, because of their low thermogenesis values (5-10% for carbohydreates, and 20-30% for proteins), and because these 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 (Hamad and Travis 2006).

Energy assimilation and life history. — The positive role of meat in the reproductive performance of female brown bears is well documented (Bunnell and Tait 1981; Hilderbrand et al. 1999; Reynolds and Garner 1987). Fruits are the second most important source of protein and energy, and are consumed by brown bears in large quantities. For example berries make 82% of the autumn diet in central Sweden (Dahle et al. 1998), and pine nuts make up to 45% scat volume in Yellowstone (Kendall 1983). Brown bears with access to abundant salmon are also reported to feed extensively on fruits (87% fecal volume, Fortin et al. 2007). Robbins et al. (2007) 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 food energy in 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 (Schaller 1998, Xu et al. 2006), 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 low nutritious diet and facing extreme environmental conditions, are expected to have poor reproductive performance

(Bunnell and Tait 1981; Ferguson and McLoughlin 2000). These factors probably contribute to the very low reproductive rates of the brown bear population in DNP (Paper III).

The Central Asian populations, which are closer to the Himalayan brown bear genetically and geographically (Galbreath et al. 2007; Nawaz 2007), have access to fruits 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 than in DNP, because these areas have wild ungulates and a variety of fruit plants. For example Schaller (1977) 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.

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FIGURE LEGENDS

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.

Fig 2. Sex differences in the diet of brown bears in Deosai National Park, Pakistan, based on scat analysis.

Fig 3. Temporal trend in probabilities of major diet categories of brown bears in Deosai National Park, Pakistan, based on scat analysis.

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	

[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]Nawaz et al. (2006).

Family	Species	Rank[1]	Frequency	Food Type	Identification source[2]	Comments
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.

Family	Species	Rank[1]	Frequency	Food Type	Identification source[2]	Comments
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.
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]

Family	Species	Rank[1]	Frequency	Food Type	Identification source[2]	Comments
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	

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
G^2	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

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 (Hewitt and Robbins 19996) and energy estimates of food items (Pritchard and Robbins 1990) 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; Nawaz 2008
Kekexili Nature Reserve, China	2	-	98 [d,e]	25.6		Xu et al. 2006
Chang Tang Reserve, China	26.2	-	73.8 [d,e]	22.8		Schaller 1998
Southern Hokkaido, Japan	72.3	17 [a]	10.7 [c,e,f]	20.9		Nomura and Higashi 2000
Northern Hokkaido, Japan	48.3	46.2 [a]	5.5 [c,d,e]	19.3		Ohdachi 1987
Western Tian Shan, Central Asia	22	55.7 [a]	20.8 [d,e,f]	21.1		Vaisfeld and Chestin 1993
Northern Tian Shan, Central Asia	60.9	20.5 [a,b]	18.6 [e]	20.6		Vaisfeld and Chestin 1993
Caucasian Reserve, Russia	35	53 [a,b]	12 [e]	23.9		Vaisfeld and Chestin 1993
Eastern Sayans, Russia	28.9	38.7 [a,b]	32.4 [e,f]	23.5		Vaisfeld and Chestin 1993
Western Sayans, Russia	34.4	54.8 [a]	10.8 [d,e,f]	24.3		Vaisfeld and Chestin 1993
Far East, Russia	23.5	43.2 [a,b]	33.4 [e,f]	25.5		Vaisfeld and Chestin 1993
Europe						
Central Sweden	43.6	26.7 [a]	29.7 [e,f]	20.1	0.96	Dahle et al. 1998; Sæther et al. 1998
North-eastern Norway	20.9	38.1 [a]	41 [e,f]	25.1		Persson et al. 2001
Nord-Trøndelag, Norway	33.3	16 [a]	50.7 [e,f]	26.6		Dahle et al. 1998
Central-south Norway[***]	25	39 [a]	36 [d]	20.5		Elgmork and Kaasa 1992
Riaño National Hunting Reserve, Spain	45.5	40.6 [a,b]	13.9 [e,f]	24.4		Clevenger et al. 1992
Cantabrian Mountains, Spain	34.1	56 [a,b]	9.9 [e,f]	24.0		Naves et al. 2006
Yugoslavia (Croatia)	29.1	68.7 [a,b]	2.2 [e,f]	22.8		Cicnjak et al. 1987
North America						
Northern Yukon, Canada	76.4	20.3 [a]	3.3 [d,e,f]	16.9	0.50	Ferguson and McLoughlin 2000; MacHutchon and Wellwood 2003; Mclellan 1994
West-central, Alberta, Canada	65.5	21.9 [a]	12.7 [e,f]	21.3		Munro et al. 2006
Banff National Park, Canada	65	25 [a]	10 [e]	21.3	0.48	Garshelis et al. 2005; Hamer and Herrero 1987
Flathead River Drainage, BC, Canada	52	29 [a]	19 [d,e,f]	22.6	0.85	McLellan 1989; McLellan and Hovey 1995
Yellowstone 1973-74, USA	80.1	6.1 [a,b]	13.8 [c,d,e]	22.7	0.62	Mealey 1980; Schwartz et al. 2006

[*] 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

Fig 1.

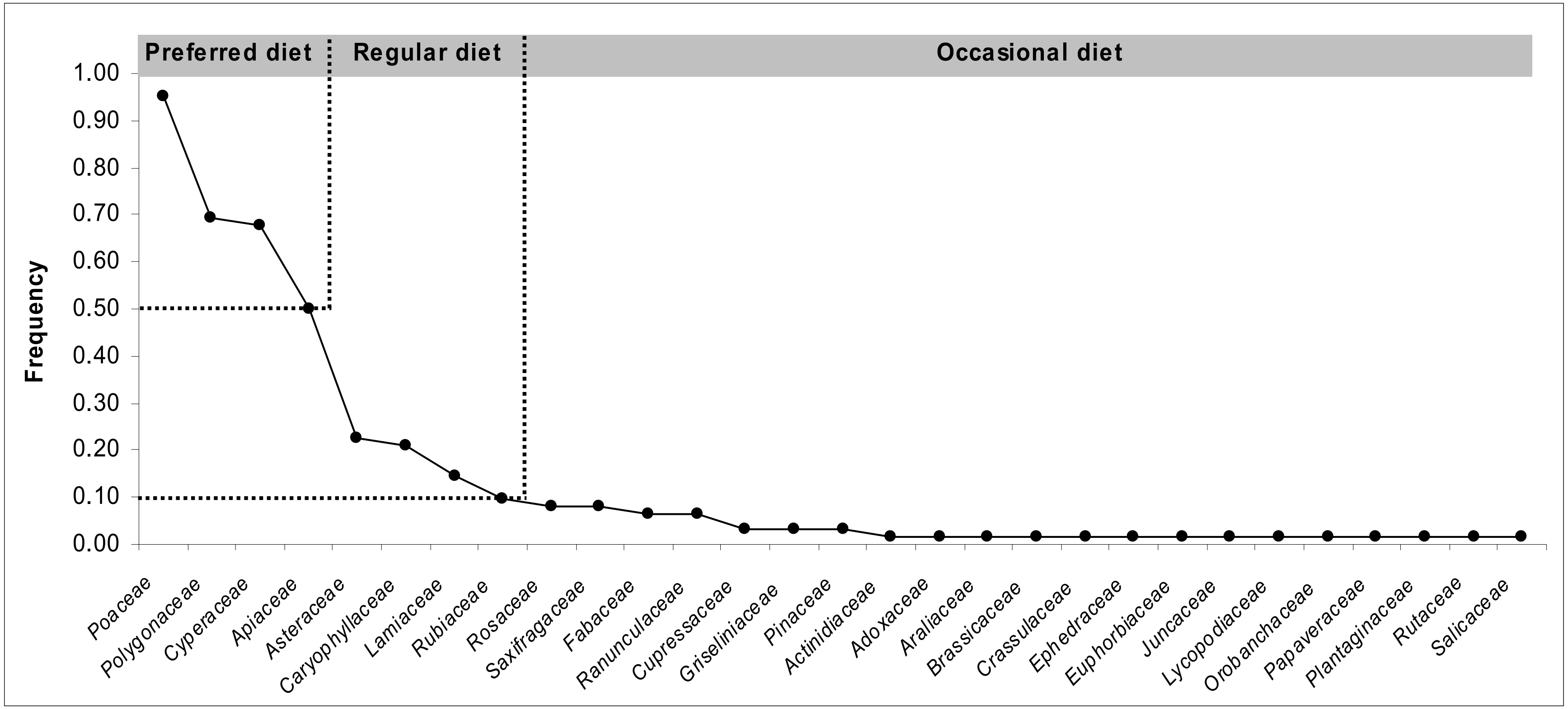
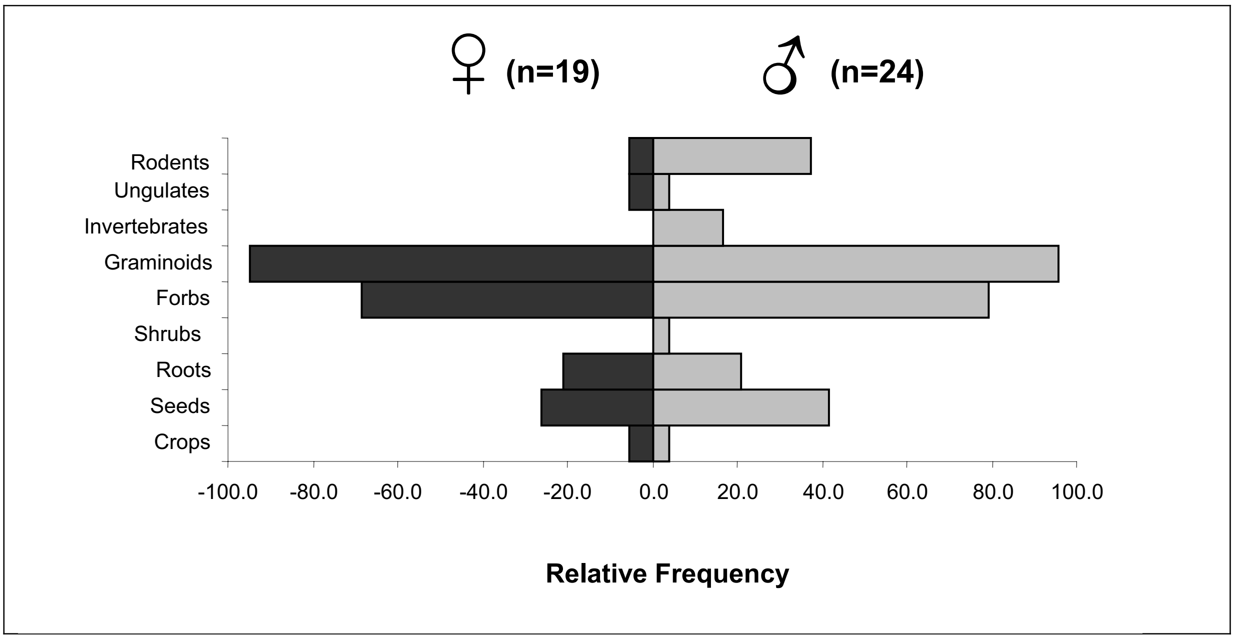
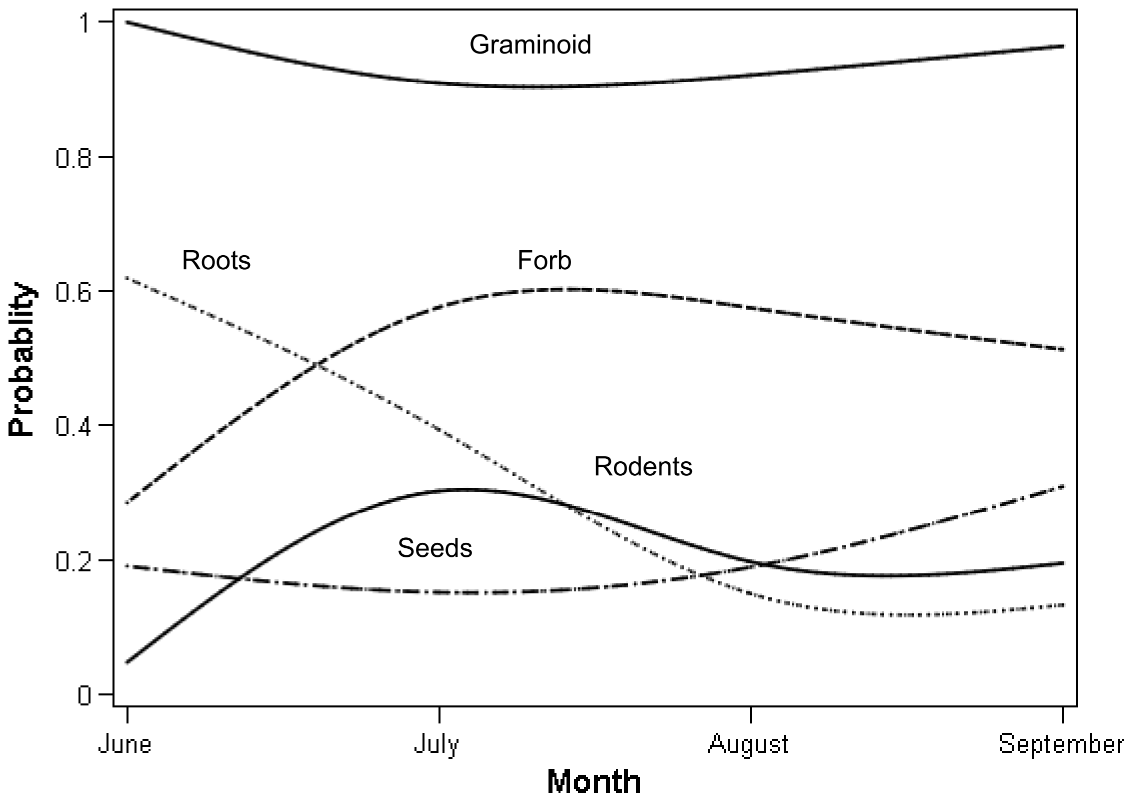

Preferred diet
Regular diet
Occasional diet
Frequency
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Poaceae
Polygonaceae
Cyperaceae
Apiaceae
Asteraceae
Caryophyllaceae
Lamiaceae
Rubiaceae
Rosaceae
Saxifragaceae
Fabaceae
Ranunculaceae
Cupressaceae
Griseliniaceae
Pinaceae
Actinidiaceae
Adoxaceae
Araliaceae
Brassicaceae
Crassulaceae
Ephedraceae
Euphorbiaceae
Juncaceae
Lycopodiaceae
Orobanchaceae
Papaveraceae
Plantaginaceae
Rutaceae
Salicaceae

Fig 2.



♀ (n=19)
♂ (n=24)
Rodents
Ungulates
Invertebrates
Graminoids
Forbs
Shrubs
Roots
Seeds
Crops
-100.0
-80.0
-60.0
-40.0
-20.0
0.0
20.0
40.0
60.0
80.0
100.0
Relative Frequency

Fig 3.



Graminoid
Roots
Forb
Rodents
Seeds
Probablity
1
0.8
0.6
0.4
0.2
0
June
July
August
September
Month

Paper VI

HABITAT SELECTION BY BROWN BEARS IN DEOSAI NATIONAL PARK, PAKISTAN, AND IMPLICATIONS FOR PARK MANAGEMENT

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Abstract

The Himalayan brown bear is a threatened species with a fragmented range in the Himalayas, yet its habit requirements are not known. We investigated habitat selection of brown bears and the impact of human disturbance factors in Deosai National Park, Pakistan.

An Ecological Niche Factor Analysis indicated that bears avoided higher elevations and steeper slopes, and showed a higher preference for more productive parts of the park (marshy, grassy, and stony vegetation types). Only 65% area of the park was vegetatively productive, with a standing crop of about 900 kg dry matter/km^2. The marshy vegetation was the most preferred habitat, probably due to its highest forage production and highest density of golden marmots. Brown bears tolerated human structures like roads and camps, but strongly avoided grazing areas with high livestock density. The habitat suitability map generally followed the biomass productivity patterns of the park. It indicated the central part as suitable, and classified half of the park, mainly peripheral areas, as not suitable for brown bears.

The vegetation and habitat suitability maps provide an objective criterion for evaluating present and future developments in the park. Until recently, the park seems to have sustained resource use by communities without significantly affecting the brown bear population or other park resources. However a large influx of livestock by nomad grazers in the last two years has become a major challenge, which needs urgent attention to continue the present brown bear population recover and to secure its habitat. We recommend monitoring of the livestock and a detailed inventory of the rangeland to understand grazing dynamics in the park and to maintain sustainable stocking rates.

Key words: ENFA, habitat selection, Himalaya, habitat suitability map, Pakistan, *Ursus arctos*

Introduction

Human persecution, increases in human populations and their activities, and habitat degradation and fragmentation have reduced populations of large carnivores in much of the world (Weber and Rabinowitz 1996; Woodroffe 2000). Protected areas can provide an important sanctuary for sensitive species, such as large carnivores, but they are often too small to provide for viable populations (Newmark 1995; Woodroffe and Ginsberg 1998). In addition, protected areas sometimes hold lower densities of important species for protection than adjacent areas used by humans (Rannestad et al. 2006) or may constitute a population sink, with the source being on the adjacent human-used lands (Swenson et al. 1986). Nevertheless, protected areas often constitute important, core habitats that allow large carnivores to better survive in mostly human-dominated landscapes (Schwartz et al. 2006). Even in protected areas, zoning is an increasingly popular approach. Zoning results in distributing the resources within a protected area among various competing interests, such as human uses and wildlife, in order to meet management goals (Hepcan 2000; Kothari et al. 1996). However reserving suitable areas for wildlife requires specialized knowledge, but managers often select areas on an ad hoc basis without a clear understanding of the ecological needs of species.

Brown bears (*Ursus arctos*) are highly endangered in Southern Asia, where mostly small, isolated populations exist in the remote and rugged mountainous areas (Servheen 1990). Similarly the Himalayan brown bear (*U. a. isabellinus*) is a highly threatened species with fragmented populations in Pakistan (Nawaz 2007). To date, almost no research has been conducted on the habitat requirements of brown bears in the Himalayan region.

When Deosai National Park (DNP) was created in northern Pakistan in 1993, a zoning plan was introduced to accommodate the resource needs of local communities and nomad grazers (HWF 1999). Although people were allowed to use resources in consumptive zones, a "core area" was designated for brown bears, which was managed as a restricted area, where public entry was not allowed. This was done because the conservation of the remnant bear population was one of the goals of the park when it was created (HWF 1999). The ecological needs of brown bears were not known at that time, therefore the demarcation of the core area was based on sightings of brown bears and subjective assessments. The brown bear population in the park is growing (Paper III), which suggests that the management has been positive for the bears. Nevertheless, livestock numbers in the park are also increasing. There have been unsuccessful attempts by the livestock herders to encroach into the core area, and new developments have been proposed for the park, including new roads, hotels, sport facilities, etc. A better understanding of the park resources and how bears respond to human activities is required to understand how these issues might affect the bear population. It is therefore very important for management of the park; a) to understand habitat preferences of brown bears, and b) to assess park resources (particularly pastures) and its spatial distribution in relation to bears. This study aims to address these questions.

Materials and Methods

Study area

DNP, about 1800 km^2, occupies part 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 tree line and vegetation is predominately herbaceous perennials, grasses and sedges.

The grazing ranges of the park are an essential resource for wildlife, particularly brown bears (Nawaz 2007). These rangelands also contribute substantially to the livelihood of local communities and nomadic groups (*Bakarwals* or *Gujjars*). About 9,000 livestock, mainly goats and sheeps, grazed within the DNP in 2004. According to the zoning plan, the south-eastern part of the park, covering about half of its area, was designated as the core area for brown bears, whereas local communities and Gujjars were allowed to continue grazing ranges in rest of the park.

Data collection

The locations of brown bear feces (hereafter referred to as sign) were used to indicate areas used by bears. We divided the study area into five blocks, and each block was searched for sign every year in order to cover most of DNP (see details in Bellemain et al. 2007). In addition to this planned collection, the DNP field staff recorded sign during their normal patrolling of the park. A total of 450 occurrences of sign were documented between 2003-2006.

Vegetation classification

We used the 28 July 1998 LANDSAT Thematic Mapper (TM) satellite image for habitat classification. A subset of the study area was made after geocorrection of the image. The image comes with seven bands (1: visible blue, 2: visible green, 3: visible red, 4: near infra red, 5:

middle infra red, 6: thermal infra red, 7: short wave infra red). The false color composites of 7, 4, 3 and 4, 3, 2 (in red, green, and blue) were useful in discriminating vegetation types in DNP.

We used a combination of supervised and unsupervised classification tools and ground control points in the ERDAS Imagine Program (Leica Geosystems, Inc.), to classify DNP into six classes; marshy, grassy, stony, rocky, water and snow (Table 1). The cloud-covered areas in the 28 July 1998 LANDSAT image, about 8%, were replaced by 30 September 2001 LANDSAT Enhanced Thematic Mapper (ETM) image.

Standing biomass assessment

To obtain an index of forage production, from standing crop (Soest 1994; Vallentine 1990), we randomly established 5 quadrats (0.5 x 0.5 m) in marshy areas, 5 in grassy and 6 in stony areas (both 1.5 x 1.5 m). All edible parts (twigs, leaves, etc) from shrubs and whole plants of herbs and grasses were clipped and stored for dry matter (DM) biomass analysis. Sampling was done in August (mid growing season) in low grazing area. We collected only palatable species, supposed to be eaten by bears and livestock. The collected samples were weighed and oven-dried for 24 hrs at 70°C in a fan-forced oven. Dry matter weight was then calculated, and biomass production per unit area was calculated.

Data preparation

We projected the map of the DNP on the UTM (WGS 84, 43N) coordinate system. Raster maps of 11 ecogeographical variables (EGV) (Table 2) were prepared in Arc GIS (ESRI Inc., 2006). Resource units (RU) were defined as 200 x 200 m pixels of raster maps (Manly et al. 2002).

We acquired elevation data from the Shuttle Radar Topography Mission (SRTM) (http://www2.jpl.nasa.gov/srtm/). For DNP, two SRTM images (N34E075, N35E075) were required, which we joined to make a subset for the study area (Fig. 1a). The areas of missing data ("voids") in the SRTM images were replaced with information from topographical maps of the Survey of Pakistan, using ERDAS Imagine Program (Leica Geosystems, Inc.). Streams were digitized from the 30 September 2001 LANDSAT image. Roads were digitized from topographical maps of the Survey of Pakistan, and were categorized as main and small roads, depending on their size and traffic volume. There was a single main road, crossing DNP in the middle, and connecting the two main towns of the area; Skardu on the east and Astore towards the southwest. This road receives public transport travelling between these towns and other villages on the way. Also tourists coming to DNP or travelling in Baltistan use this road. There were two minor roads that connect Matyal Village and the Gultari/Minimerg Valley to this main road. These are smaller roads with considerably low traffic volume, because these are generally used by vehicles bringing supplies to these villages or transporting agricultural products.

Locations of camps belonging to nomad and local livestock herders and seasonal hotels were recorded with a GPS receiver. During the vegetation surveys in 2002-2003, livestock grazing pressure in Deosai was documented from the proportion of plants grazed in quadrats and the park area was divided into three grazing impact zones; high, medium, low (Nawaz et al. 2006). We calculated topographic ruggedness index by using the TRI Arc Macro Language (AML) code in Arc/Info Workstation (Arc 9.2 ESRI 1982-2006). The TRI is a measurement developed by Riley et al. (1999) to express the amount of elevation difference between adjacent cells of a digital elevation grid. The TRI has been used to explain habitat selection of large mammals (Nellemann and Cameron 1996; Nellemann and Reynolds 1997; Vistnes and Nellemann 2001) including brown bear (Nellemann et al. 2007). In the present study TRI and the slope map were quite identical in pattern and also highly correlated (r: 0.87), we therefore did not use TRI in further analysis.

Data analysis

We performed Principal Component Analysis (PCA) involving 11 EGVs (Table 2) to determine the spatial relationship among landscape components. We investigated spatial pattern at locations of bear sign by calculating mean center, directional distribution, and average nearest-neighbor distance (O'Sullivan and Unwin 2003). The ratio between the observed and expected mean nearest-neighbor distances indicates tendency towards clustering if the value is <1; a larger value means that events are evenly spaced. We summarized counts of bear sign in a grid of 4x4 km, investigated spatial autocorrelation at this scale by computing Moran's I, and calculated relative density of sign using kriging interpolation (O'Sullivan and Unwin, 2003). These statistics helped determining whether bear movement in the landscape was random or concentrated in particular areas.

We used Ecological Niche Factor Analysis (ENFA) to investigate habitat preferences of the brown bear in DNP. ENFA, developed by Hirzel et. al. (2002), is based on Hutchinson's (1957) concept of niche, defined as a hypervolume in the multidimensional space of habitat characteristics. It is a multivariate method that first extracts one axis of marginality and then several axes of specialization. The marginality axis measures the difference between the conditions used on average by the species and the mean available habitat. The coefficients of the marginality factor determine magnitude and direction (preference, avoidance) for each EGV. The specialization factor is calculated as the ratio of the global variation in an EGV to the variation in the part utilized by the focal species. It is a measure of the width of the niche within available habitat. The higher absolute coefficients (sign is arbitrary) indicate a restricted range of focal species for that EGV (Hirzel et. al. 2002, Basille et.al., In press). The biplot of an ENFA is a useful visualization of the ecological niche of a species. It projects used and available resource units in the ecological space on the plane defined by the marginality axis and one specialization axis. All EGVs are projected by arrows on this plot, and their length and direction express their influence on the position and volume of the ecological niche (Basille et.al., In press).

We used locations of bear sign as the response variable, and normalized some EGVs by square-root transformation. All analyses were carried out with the Adehabitat package (Calenge 2006) in R-software (R Development Core Team, 2006). A randomization test was performed to test the significance of marginality and the first eigenvalue of specialization. One thousand sets of 450 localizations were distributed randomly over the study area. Marginality and specialization were computed for each set of random locations and compared with actual values (Manly 1997, Basille et al. in press).

Habitat suitability mapping

We used Mahalanobis distance statistics (Clark et al. 1993) to compute a habitat suitability map. It is a measure of dissimilarity between the average habitat characteristics at each resource unit (pixel) and the mean of habitat characteristics estimated from animal locations. Thus smaller distances represent better habitat. Assuming multivariate normality, squared Mahalanobis distances have a Chi-square distribution with n-1 degrees of freedom (n = number of EGVs). The Adehabitat package in R (Calenge 2006) allows computing a map with continuous gradient of suitability (pixels represented by p-values ranging 0-1) from squared Mahalanobis distances. This gradient of suitability coveys more information, yet for managers it is more convenient to work with few classes (suitable, unsuitable, etc). Hirzel el. al. (2006) noted that a continuous scale is often misleading, because in a real environment the suitability index may not be linearly proportional to the probability of use; real curves may have staircase or exponential shapes. They suggested computing a curve of the ratio of expected-to-predicted frequencies of evaluations points. This curve provides insight into accuracy of the habitat suitability map, and also provides an objective criterion for choosing thresholds for reclassifying suitability maps into few classes.

We used all EGVs in Table 2, except slope, because it was correlated with elevation (r = 0.51). We divided the habitat suitability map into 10 classes (with 0.1 intervals), and calculated predicted-to-expected ratios (F_i) for each class (Hirzel et al. 2006):

$F_i = \dfrac{p_i}{E_i}$, where p_i is the predicted frequency of evaluation points in class i, and E_i is the expected frequency as expressed as relative area covered by each class.

We plotted F_i against class intervals (Hirzel et al. 2006) and reclassified the suitability map into three classes (poor, suitable, and high quality) by choosing threshold points from the F_i

curve. F_i = 1 indicates a random model when presences are equal to expected by chance. We choose this point as the boundary between poor ($F_i \leq 1$) and suitable ($F_i > 1$) habitats (Hirzel et al. 2006). The second boundary for a high quality habitat was selected at $F_i \geq 2$, when the curve became steeper after a plateau.

The predictive power of the habitat suitability map was evaluated by the Boyce Index (Boyce et al. 2002; Hirzel et al. 2006), calculated as Spearman rank correlation coefficient between F_i and i . The positive values in the Boyce Index (range: -1 to 1) indicate good prediction power of the habitat suitability map, zero means a random model, and negative values indicate an incorrect model.

Results

Description of the landscape

The 15% of the DNP was classified as marshy, 27% as grassy, 23% stony, 30% rocky, 5% permanent snow and 1% water (Table 1, Fig. 1b). The standing plant biomass of the park occurred on marshy, grassy and stony areas, with 35% of the area (rock, snow, water) being vegetatively unproductive. The average standing biomass of the park was 900 kg DM/km^2. Marshy areas contributed 56% of the total biomass, followed by grassy areas with 34%.

The central part of the DNP is relatively flat (0-10° slope) at elevations between 3400-4000 m, whereas the peripheral areas are steeper (up to 50° slope), with elevations up to 5300 m. The first Principal Component (PC) explained 30% of the variation in the data, and showed that elevation, slope and rocky areas were highly correlated (Fig 2). This component can be considered as a *productivity component,* as it contrasts between productive areas (marshy, grassy and stony vegetation types) and unproductive parts of the park. It indicated that productive areas were associated with lower elevations and occupied flatter terrain. This means that the central part of the park is productive, whereas the peripheral parts are predominantly rock and snow. The second PC, which explained 14% of the variation, showed that camps were associated with roads and that both were closer to rivers. The higher levels of grazing impact also were related to roads and camps. In the first PC, roads and camps were linked with lower elevations, which means that human structures are situated in the productive part of the park. In the second PC, marshy vegetation also was associated more with stony vegetation than grassy vegetation areas.

Spatial pattern of bear sign

The mean center of the bear sign locations (X: 539034, Y: 3871490) was located in center of the park (Fig. 1c). Average nearest-neighbor distance (1.68) suggested dispersion in the data ($P < 0.01$), and directional distribution showed an east-world trend in the data (rotation of long axis: 78.35°). In contrast to the pattern depicted by individual locations, counts of sign in 4x4 km grid cells indicated strong autocorrelation (Moran's I: 0.09) and a tendency for clustering. This suggested that the bear use of the landscape was not random, and bear sign was concentrated in the central parts of the park (Fig. 1c). The "Black Hole" and "Bowl" areas, in the central part of the park, had the highest density of sign (> 20 per grid cell), which is in agreement with the highest density of brown bears in this area (Paper III).

Habitat selection

Bear use of habitat differed significantly from random, as indicated by randomization tests carried out on marginality and the first axis of specialization ($P < 0.001$, for both tests). The global marginality was 2.435, signifying that the niche of the brown bear was different from the mean of available conditions (Fig. 3). Elevation and slope had the largest coefficients for marginality, indicating strong avoidance of higher elevations and steeper slopes (Table 3). Bears preferred marshy, stony and grassy vegetation types, and avoided rocky areas. Interpretation of EGVs that were measured as distances from objects (like distance to streams, roads, camps) is tricky, because negative coefficients of the marginality factor for these EGVs would mean the occurrence of bears was in the proximity of these objects. Large negative coefficients for human disturbance factors (distances to roads and camps) therefore suggested that bears were tolerant to these structures. The marginality factor also indicated that the bears occupied the proximity of streams. There was a negative relationship between the level of grazing impact and the bears' habitat use.

The specialization factor implied that the ecological niche of brown bears in Deosai was much narrower than the available variation in habitat components. Elevation, slope and grazing impact were the most prominent variables affecting this factor. As these variables also had negative coefficients on the marginality axis, the higher values on the specialization axis suggested a mean shift towards lower values. Thus, bears utilized narrower ranges of available variation in these variables towards their lower range. For example, the slope of the study area ranged between 0°-50°, yet the majority of the bear sign (89%) was located in areas with <15° slope (which covered 64% of the total slope surface).

Habitat suitability map

The habitat suitability map, based on Mahalanobis distance (Fig. 1d), indicated that the DNP offers a range of bad to excellent habitat to brown bears. F_i values ranged between 0.4-2.5 (Fig. 4), and the Boyce Index (Spear man r: 0.98, P < 0.01) indicated good prediction power of the suitability map. About 49% of the area was classified as poor habitat, 39% was suitable, and 12% of the area constituted high quality habitat. The suitability map generally followed the productivity contour of the park, although the northeastern part of the park, with good productivity, received a low suitability value. This was probably due to the high grazing pressure there. The central part of the park was mapped as the most suitable for brown bears, with the peripheral parts as least suitable.

Discussion

The brown bear is omnivorous and, although feeding habits are complex, plant base classification provides a useful means for describing bear habitat, as in other mammals (Craighead et al. 1995; Morrison et al. 2006). However, habitat and ecological niche are by definition multivariate concepts (Hutchinson 1987; Hirzel et. al. 2002). Multivariate methods as ENFA or Mahalanobis distances allow including several variables (elevation, slope, human disturbance, vegetation type) simultaneously in analyses and therefore provide a comprehensive understanding of the habitat selection.

Himalayan brown bears are known to occupy higher elevations, for example in the Karakoram Range they occupy areas >5000 m. The avoidance of high elevations (>4500 m) in Deosai is probably because these areas are just rock and ice. Similarly, brown bear habitat in Neelam and Gurez valleys (Nawaz 2007) is much steeper than in DNP, but those slopes are covered with forest. Habitat selection by brown bears in DNP therefore is related primarily to biomass production, which we indexed by measuring standing crop. Thus almost all Himalayan alpine meadows can be considered as suitable, or potentially suitable, habitat for bears, except for the rock- and ice-dominated areas.

Marshy vegetation was the most selected habitat, probably because it had the highest vegetative productivity. Moreover, the abundance of golden marmot (*Marmota marmota*), which is the main meat source for brown bears, is also related to vegetation types. Indeed, they occur with higher density in marshy areas (1.4 times higher density than in grassy and stony vegetation, Paper V). Diet analyses (Paper V) indicated that brown bears in Deosai consume a wide range of plant species, with a higher preference for graminoids, which is a dominant plant group in marshy areas. The higher preference for stony vegetation than grassy vegetation is counter-institutive, because the grassy vegetation habitat type was more productive vegetatively. However, stony areas have a marmot density similar to grassy areas (18 and 20 colonies per km^2, respectively, Paper V), and stony areas were more closely associated with marshes (r: 0.327) than grassy areas (r: 0.016). The majority of the known brown bear bedding sites are located on stony slopes at the banks of marshes. These locations also provided a good visibility of a broader landscape, which may explain the higher density of sign (scats) there.

The selection of areas close to roads and camps could be a byproduct of the proximity of these structures to productive habitats (marshy, stony, and grassy vegetations). The ENFA therefore suggests that brown bears tolerate human presence, when it was within a suitable habitat. The continuous monitoring in the park since 1993 has reduced poaching and ensured that people living in camps (livestock herders) or visitors do not harass bears. Elusive species can occupy areas close to human presence (Zimmermann 2004) if they do not associate human activity with threat. An activity pattern study (Nawaz and Kok 2004) and diet analyses (Paper V) showed that fish was not a substantial component of the brown bear's diet in DNP. The presence of bears near streams, as indicated by ENFA, is probably due to the positive correlation between streams and productive habitats (r: -0.313 between marshes and distance to streams).

The habitat suitability map depicted the central part of the park on either side of the central river (Barapani) as equally suitable for bears. The vegetation map also confirmed that both areas were almost equally productive. However the density of bear sign was relatively higher on the eastern side of the Barapani River, particularly in the Black Hole and Bowls (Fig. 1). We propose three possible reasons; 1) proximity of this eastern area to highly rugged terrain, which, although unproductive, provides escape terrain in case of danger or disturbance, 2) there are no human structures (road, camp, grazing) at all in this area, and/or 3) this area has been managed as a restricted area for the public since the inception of the park and human presence is therefore very low.

The vegetation and habitat suitability maps are the useful outcomes of this study, which can be used as decision making instrument for evaluating future developments within the park. Using these tools, we also can evaluate the effectiveness of the original zoning plan of the park. The core area for bears in the original zoning plan (HWF 1999), covering about half of the park, encompasses 50% poor, 34 suitable and 14% high quality habitat. A major part of the core area (68%) has productive vegetation types, and appears to be adequate for the requirements of the present brown bear population. However, a moderate level of grazing and the presence of camps in the western part of the core area, suggests that a gradual encroachment of human activities is occurring. This needs to be addressed to secure the quality of bear habitat in the DNP.

Conclusion and implications

Resource selection is related to reproductive success in animals (McLoughlin et al. 2006), therefore an analysis of habitat helps identifying resources critical for survival and reproduction of a species. The brown bear population in DNP has a very low reproductive capacity (Paper III), which complicates its conservation efforts. A time budget study indicated that brown bears in Deosai spend most of a day foraging (67%), mainly grazing (96%) and the rest in capturing rodents (Nawaz and Kok 2004). A diet analysis (Paper V) also showed that vegetation dominated the diet. These observations support the findings of the ENFA that the relatively dense vegetation of marshes was the most preferred habitat, and probably explains the low reproductive rates in the population, because reproductive success is related to the amount of meat in the diet in bears (Bunnell and Tait 1981; Hilderbrand et al. 1999). It also highlights the importance of marshy areas, particularly of Black Hole and Bowls (Fig. 1), as critical habitats for bears.

The brown bear is the flagship species of DNP and its protection was the core reason behind the park's establishment (HWF 1999). The habitat requirements of the brown bear should remain the key element in the management strategy for the park resources. The habitat suitability map indicated that brown bear habitat, which occupied the central part of the park, is rather contiguous and not fragmented. This central part of the park has been attractive for many development interventions in past. For example, proposals to establish a polo ground and constructing a new road (passing through the high bear density area) to access Karabosh Village probably would be detrimental for the bears.

DNP was established using the community participation approach (HWF 1999), which aimed to engage communities in conservation efforts by recognizing their rights within the park and also sharing park benefits (revenues, etc) with them. Therefore securing the livelihoods of the local communities, which is largely dependant on grazing, without compromising ecological integrity is the second important element of the park management. Until now the park seems to have been successful on both fronts, because the brown bear population is increasing while the communities are grazing within the park (Paper III).

Among the human disturbance factors, grazing pressure was the strongest factor affecting habitat suitability of the park. The park' range seems to have been able to sustain the level of grazing pressure in the past, because only 19% area was indexed as heavily impacted and avoided by bears. However livestock numbers have increased alarmingly in the last two years, particularly due to an influx of Gujjars. About 8000-9000 livestock were brought in by Gujjars in 2007, compared with approximately 5000 in 2003. The primary reason for this unprecedented increase in numbers was the careless sale of grazing permits by the Northern Areas Forest and Wildlife Department. Though a detailed inventory of the rangeland would be required to understand grazing dynamics of the park and to determine impacts on species diversity in high grazing areas, it is obvious that, with only 65% of the area being vegetatively productive, DNP cannot support this large stock without impacting brown bears and other important resources of the park.

Because poaching and other threats are under better control (Paper III), we see range and livestock management as the key management problem for future; this challenge can put the success of park management and population recovery of brown bears at risk. The expansion in livestock numbers will likely result in an expansion in their range, and may boost management challenges by increasing human-bear conflicts. Brown bears seldom attack humans and attacks on property also have been rare in past, probably due to their low density and a general segregation between areas of high bear density and high grazing density. Increased human encroachment into the core area of the growing bear population can potentially spawn more conflicts.

The third important mandate of the park is recreation and education. The ENFA indicated that bears are tolerant to the present level of activity on roads. Therefore promotion of carefully managed tourism should be acceptable. However intensive nodes of tourist-related structures like hotels or camping facilities should be limited to the peripheral areas (Ali Malik Top and Sheosar Lake). Limited guided tours into the bear core areas like "Bowls" (area along the left bank of Barapani River) should also be considered, if they are strictly managed by the staff of the wildlife department. It should promote awareness and education among visitors, which hopefully would promote conservation efforts.

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Table 1: Vegetation classes in Deosai National Park, Northern Areas, Pakistan, their spatial extent, and biomass production.

Vegetation type	Description	Area (km^2)	Biomass (kg dry matter/km^2)
Marshy vegetation	Prevalent in low-lying areas and depressions. It is dominated by various species of *Poa* and *Carex*, and *Aconitum violeceum*. Other common species of this habitat are *Veronica anagalis-aquatica, Rhodiola heterodonta, R. tibetica, Euphrasia densiflora, Lamatogonium coeruleum, Pedicularis pyramidata, Aconitum heterophyllum, Thalictrum alpinum, Primula macrophylla, Saxifraga flagellanis sub sp. stenophylla, Minuartia biflora,* and *Sausseria atkinsonii*.	262	3919.0
Grassy vegetation	Generally associated with flat or undulating areas, dominated by *Poa* species. Other associated herbs include *Bistorta affinis, Leontopodium leontopodinum, Oxytropis cashmiriana,* and shrubs include *Tanacetum falconeri, Potentilla grandiloba, Artemesia* spp., *Aster falconeri*, etc.	475	1306.6
Stony vegetation	The substrate is stony, dominated by herbs like *Saxifraga flagelaris*, *Oxytropis cashmiriana, Oxyria digyna, Lagotis kachmiriana, Aconogonon rumicifolium*, and shrubs like *Sausserea falconeri, Senecio analogus,* and *Androsace baltistanica*.	413	446.0
Rocky	Rocky or gravel areas that are generally devoid of vegetation or have a sparse cover of plants such as *Sorosaris dysaie, Saussuria gnaphalodes* and *Saxifraga jacquemontiana*, *Aster flaccida*, *Rhodiola wallichiana,* and *Primula macrophylla*.	526	0
Water	Lakes and streams	12	0
Snow	Areas of permanent snow	81	0

Table 2: Ecogeographical variables (EGVs) used in the Ecological Niche Factor Analysis of brown bear habitat and Mahalanobis distance suitability map in Deosai National Park, Northern Areas, Pakistan. Each variable was represented by a raster map of 200 m pixel size, called a Resource Unit (RU).

EGV	Code	Description
Marshy vegetation	marsh	Proportion of marshy vegetation in each RU
Grassy vegetation	grass	Proportion of grassy vegetation in each RU
Stony vegetation	stone	Proportion of stony vegetation in each RU
Rock	rock	Proportion of rocky vegetation and permanent snow in each RU
Elevation	elevation	Digital elevation data from Shuttle Radar Topography Mission (SRTM)
Slope	slope	Slope in degrees calculated by Spatial Analyst extension in Arc GIS.
Distance to stream	river	Linear distance from streams calculated by Spatial Analyst extension in Arc GIS.
Grazing impact	grazing	Livestock grazing pressure in DNP; 1: low, 2: medium, 3: high
Distance to main road	mroad	Linear distance calculated by Spatial Analyst extension in Arc GIS. Classified as; 1: 0-500m, 2: 500-1000 m, 3: 1000-2000 m, 4: 2000-3000 m, 5: 4000-5000 m, 6: > 5000m
Distance to small road	sroad	Same as above
Distance to camps	camp	Same as above

Table 3: Results of the Ecological Niche Factor Analysis of brown bear habitat in Deosai National Park, Northern Areas, Pakistan, with locations of bear scats as the response variable. Positive values on the marginality factor indicate preference, and negative values mean avoidance.

EGV	Marginality	Specialization 1	Specialization 2
Marshy vegetation	0.270	0.155	0.186
Grassy vegetation	0.087	0.062	0.036
Stony vegetation	0.277	0.096	0.108
Rock	-0.294	0.037	0.204
Elevation	-0.531	0.519	0.451
Slope	-0.490	0.446	0.078
Distance to stream	-0.272	0.067	0.048
Grazing impact	-0.157	0.529	0.264
Distance to main road	-0.283	0.184	0.231
Distance to small road	-0.071	0.369	0.147
Distance to camps	-0.225	0.199	0.745

Figure Captions:

Fig. 1. (a) A digital elevation model showing elevation range (3400-5387 m) in DNP. The 3-D view was produced by overlaying elevation layer on a hill shade map for better presentation of the geomorphology of the area. **(b)** Vegetation map, differentiating vegetation types in DNP. Black, dark gray, medium gray and light gray areas represent marshy, grassy, stony and rocky vegetation types, respectively. Water and permanent snow areas are shown as white. **(c)** Relative density of bear sign in DNP, darker gray shades showing higher densities. Gray surface was calculated by kriging interpolation using counts of bear sign in 4x4 km grids. Mean center (black point) and directional distribution as standard deviation ellipses are shown. **(d)** Habitat suitability map for brown bears in DNP. The probability distribution is based on Mahalanobis distances between the available resources and the mean of habitat characteristics used by brown bears.

Fig. 2. Loading plot of the first two Principal Components, depicting the relationships among 11 ecogeographical variables in Deosai National Park, Northern Areas, Pakistan. "barplot" of the eigenvalues. A barplot of the eigenvalues is shown as a small insert on top-right coner.

Fig. 3. Biplot of the Ecological Niche Factor Analysis of brown bear habitat in Deosai National Park, Northern Areas, Pakistan. The light gray area represents the available habitat and the dark area corresponds to the ecological niche of the brown bear (used area). The plane consists of marginality on the X axis and the first specialization on the Y axis. Ecogeographical variables are projected by arrows. The white dot corresponds to the barycentre of the niche. The distance between this point and the barycentre of available conditions (intersection of the two axes) represents the marginality of the niche within

available habitat.

Fig. 4. A plot of predicted-to-expected ratios (F_i) of evaluation points against 10 habitat suitability classes. The F_i curve shows a monotonic increase, suggesting good prediction power of the suitability map. The solid horizontal line ($F_i = 1$) is the curve of a completely random model, which makes boundary between poor ($F_i \leq 1$) and suitable ($F_i > 1$) habitats.

Fig. 1.

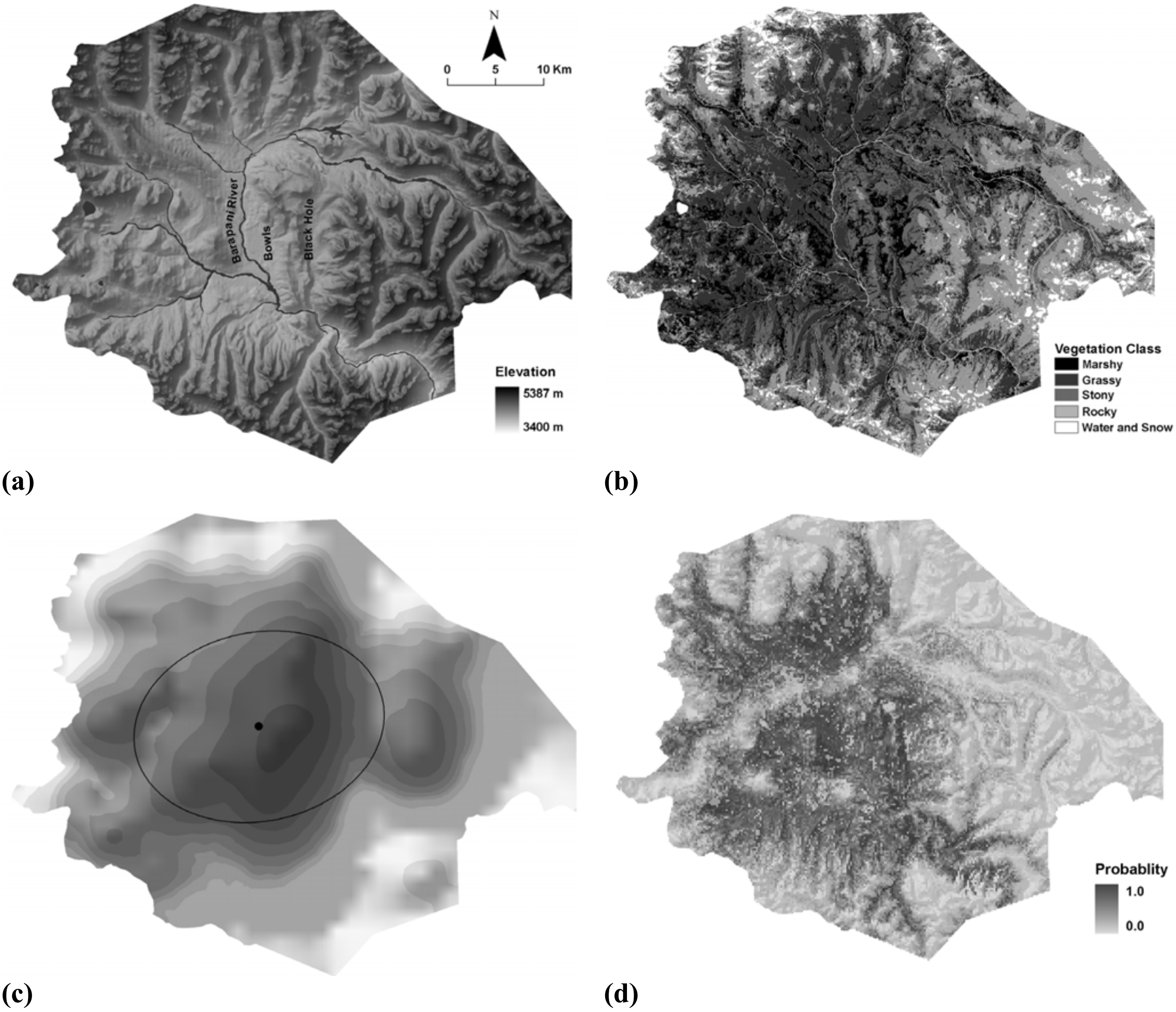

N
0
5
10 Km
Barapani River
Bowls
Black Hole
Elevation
5387 m
3400 m
(a)
Vegetation Class
Marshy
Grassy
Stony
Rocky
Water and Snow
(b)
(c)
Probablity
1.0
0.0
(d)

Fig. 2.

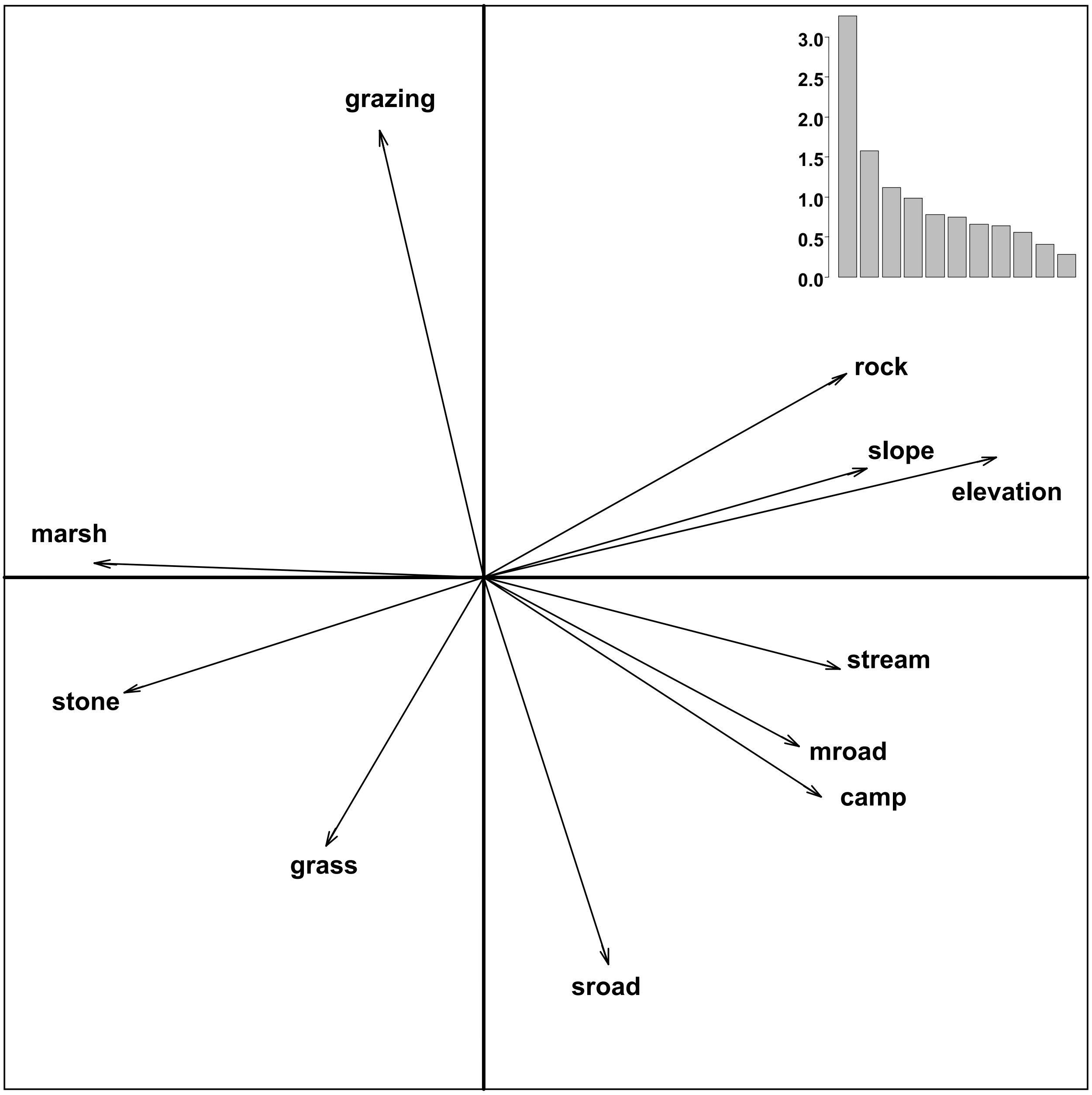
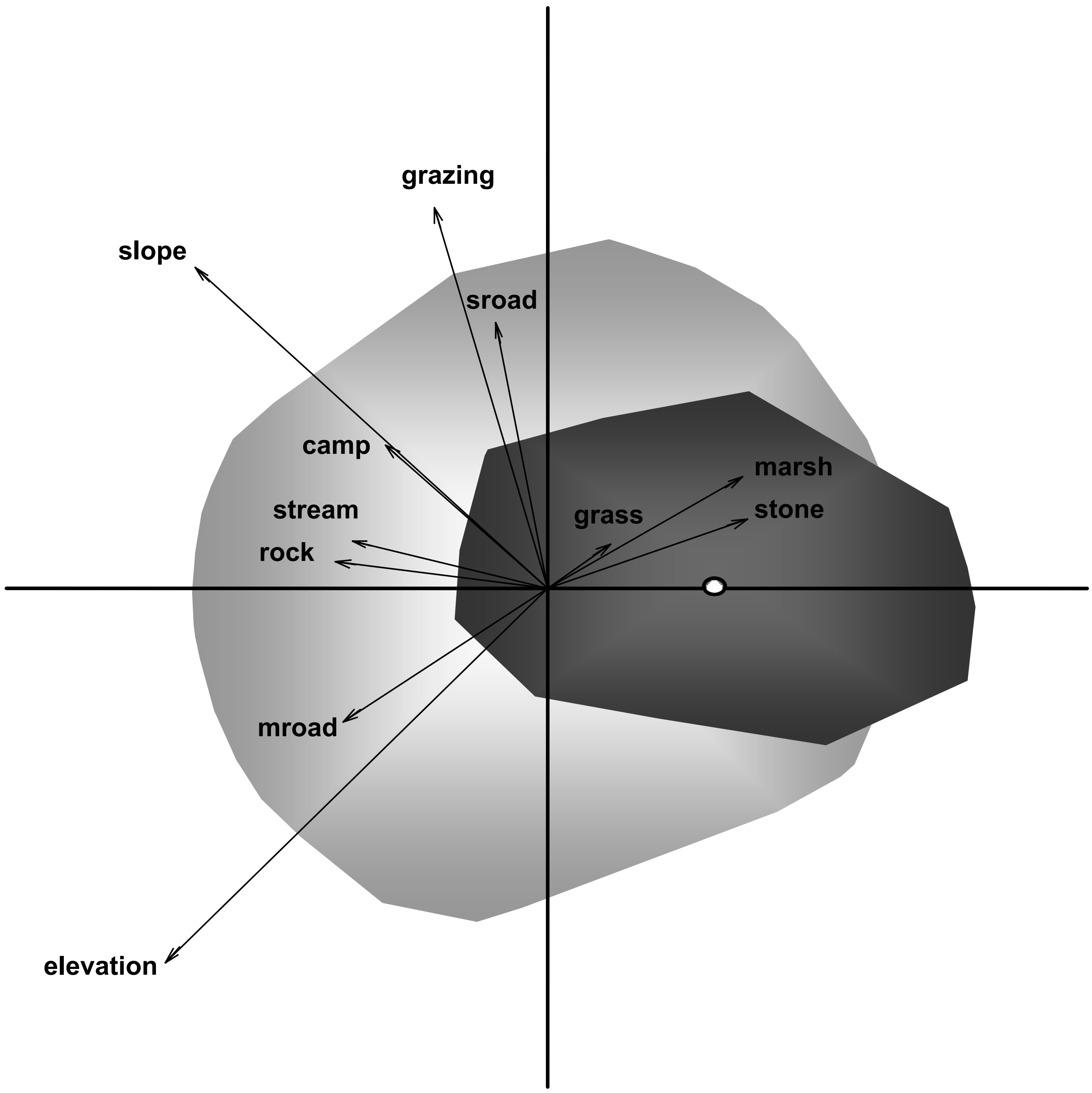

3.0
2.5
2.0
1.5
1.0
0.5
0.0
grazing
rock
slope
elevation
marsh
stream
stone
mroad
camp
grass
sroad

Fig. 3.


grazing
slope
sroad
camp
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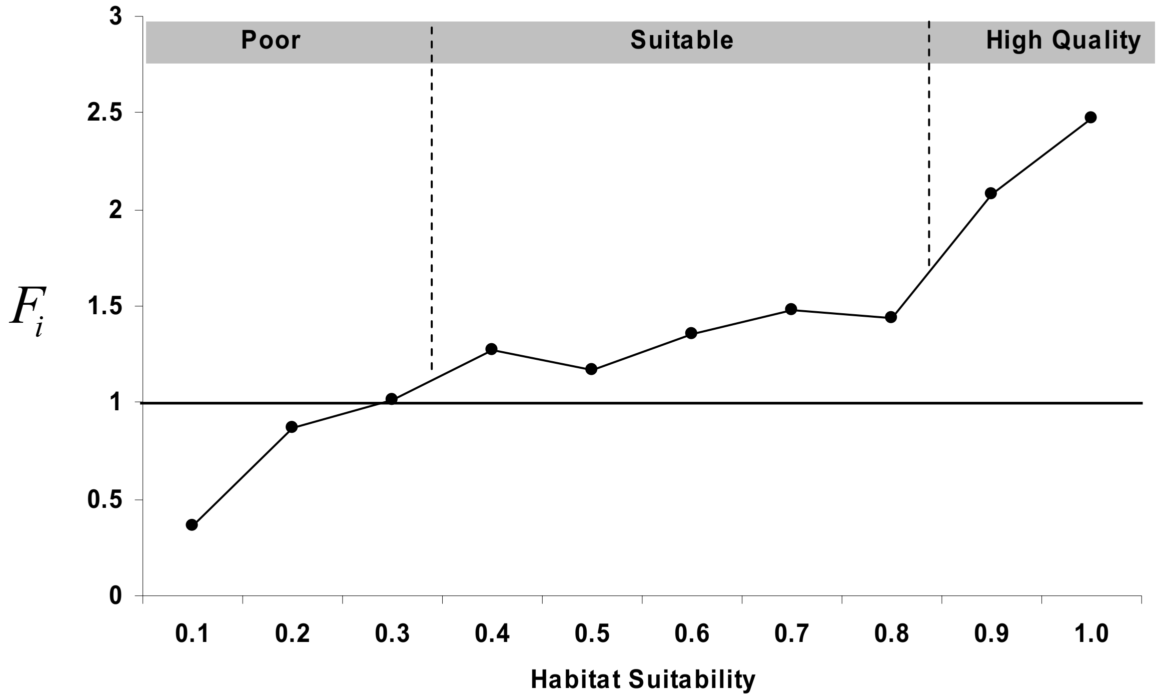
stream
grass
stone
rock
mroad
elevation

Fig. 4.


Poor
Suitable
High Quality
3
2.5
2
1.5
1
0.5
0
F_i
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Habitat Suitability

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