Ecology and Conservation of Persian Leopard

Panthera pardus saxicolor in northeastern Iran


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by:

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Abstract

The Persian leopard is the largest cat in west Asia, and is considered to be endangered – poaching and habitat degradation are thought to be responsible. The rugged mountains and high altitudes it favours present considerable challenges for scientists, and it is consequently one of the least known subspecies. I have addressed this through exploring the subspecies' fundamental ecology across three national parks in northeastern Iran. I used satellite telemetry, camera trapping, genetic analysis and questionnaire surveys. The perceived role of leopards in livestock depredation was negligible compared to that of wolves. This was associated with relative tolerance of leopards compared with wolves; but the economic loss associated with predator damage did appear to influence peoples' attitudes. I also estimated a mean home range of 103.4 ± SE 51.8 km^2 for resident males which is larger than what has been observed in other studies on Asian leopard. Five out of six of leopards spent 17.9% of their time outside the national park, among human communities. The kill rate was quantified as 3.7 ± SE 0.5 medium-sized prey/month per leopard, which is higher than reported by previous studies. Surprisingly, considering the subspecies' reported low density based on previous studies, I found relatively high population densities, varying between 4.01 ± SE 1.98 and 8.02 ± SE 2.67 individuals/100 km^2. The number of adult leopards detected in Tandoureh (30 individuals) was larger than identified during comparable surveys at any other site globally. Persian leopards exhibited moderately high genetic diversity at six microsatellites ($A_N = 7.45$, $H_O = 0.69$, $H_E = 0.75$) and low haplotype diversity ($Hd = 0.198$) across three closely related haplotypes in NADH-5 gene. There was a weak evidence of spatial population partitioning. My research findings highlight the key role of mountainous ecosystems as refugia in supporting a high density of apex predators. Although land sharing is an inevitable solution for coexistence across Asian crowded montane landscapes, sparing mountains with improved law enforcement is encouraged for large cat conservation in Asian mountains. Finally, my thesis raises new hope for leopard viability as well as highlights the potential capacity of the Kopet Dag Ecoregion as a significant conservation unit for leopards.

Contents

Declaration

This dissertation is the result of my own work as the principal investigator and includes nothing which is the outcome of work done in collaboration except where specifically indicated below.

This dissertation is 35468 words long exclusive of bibliography, appendices, figures and tables

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Mohammad S. Farhadinia – December 2017

This work is dedicated to my wife, son and mother.

Chapter 1:

General introduction

General introduction

In this chapter, I describe the background and scope of this thesis. Then, I present an overview of the key research questions addressed in chapters. A fuller account of their theoretical and empirical contexts is presented in the relevant chapters.

1.1. Large predators: function, rarity and science

Large apex predators usually show slow reproductive rates and development, extended parental care, sparsely populated territories, and a propensity towards infanticide and reproductive suppression (Wallach et al., 2015). Equally important, two oppostite regulaltory processes frequently shape their demography. Resource availability, notably prey density is the main “bottom-up’ process affecting carnivore density (Karanth et al., 2004; Sharma et al., 2015). Conversely, ‘top-down’ processes, mostly diseases (Gilbert et al., 2015; Kissui and Packer, 2004) or enemies, such as humans or intra-guild competitors (Kortello et al., 2007; Palomares and Caro, 1999) operate to shape predators’ populations. As a consequence of these opposite regulatory processes, many of large carnivores face multiple serious threats to their survival. Currently, 77% of large carnivores have declining populations and 61% are classified as threatened by the International Union for Conservation of Nature (Ripple et al., 2014).

Many apex predators impose top-down regulation on the density of their prey and smaller meso-predators (Ripple et al., 2014; Wallach et al., 2015). The loss of these apex consumers can be associated with dramatic effects of trophic downgrading on ecosystem process, function, and resilience (Estes et al., 2011). Subsequently, their loss can catalyse co-extinctions of organisms whose functionality, directly or indirectly, depend on irreplaceable ecological interactions and functional roles such as parasitism, predation, commensalism and mutualism, provided by the megafauna (Galetti et al., 2017).

Carnivores are among the most intensively studied mammalian orders, but with variation among species in research effort (Brooke et al., 2014). Felidae, particularly the larger species of the genus *Panthera*, have been subject to a tremendous amount of research effort in the past five decades, including the common leopard *P.pardus* (Brodie, 2009; Brooke et al., 2014). The conservation outcomes for leopards, however, do not, arguably, reflect this effort. This is at least partially because of the mismatch in interests, priorities and capacities of pure science and conservation (Balme et al., 2014). And although leopard research is increasing, research effort is focused on the subspecies with the largest remaining range. Subspecies with smaller geographic ranges, often those that are most in need of urgent attention have been neglected (Jacobson et al., 2016). The current thesis will be a benchmark in expanding the current research efforts for leopards across a region where the species is highly conservation-dependent, i.e. west Asia.

1.2. From “behavior ecology” to “conservation biology”

Behavioral ecology and conservation biology have developed largely independently. Behavioral ecology has remained principally an area of fundamental research while conservation biology is shaped by increasing concern about accelerating threats to biodiversity (Gosling and Sutherland, 2000). Conservation biology emerged from population ecology, population genetics and systematics (Caro, 1999), and is concerned with populations, whereas behavioral ecology is principally concerned with individual variation in behavior (Caro, 2007). Despite obvious areas of common interest, there has been little admixture between the behavioral ecology and conservation biology (Caro, 2007, 1999; but see Gosling and Sutherland, 2000). Nonetheless, practical and conceptual issues in conservation biology can benefit from a knowledge of behavioral ecology (Macdonald, 2016).

The first and most important step for crossing the split is to explore how a conservation issue affects behavioral responses (Berger-Tal et al., 2016; Caro, 2007). It is less likely that progress can be made for real world conservation action without bridging between behavioral ecology and conservation biology (Gosling and Sutherland, 2000). In this thesis, I have adopted this holistic approach for an endangered large predator, i.e. Persian leopard *P. p. saxicolor*.

1.3. About leopard *Panthera pardus*: the largest extant cat in west Asia

1.3.1. Felidae: evolution and phylogeny

Currently 38 species of felids are recognized; they occur everywhere with the exception of Australasia and the polar regions (Kitchener et al., 2017). The origins of the family Felidae are relatively uncontroversial - the gap between the earliest unequivocal felids and their ancestors among Carnivoramorpha is relatively substantial (Werdelin et al., 2010). The first felid-like carnivores appeared in the Oligocene, approximately 35 million years ago (Mya). Living cat species (subfamily Felinae) originated in the late Miocene and evolved into one of the world's most successful carnivore families regarding range, number and shaping communities (Johnson et al., 2006).

The Felidae is composed of two distinct evolutionary lineages, the modern cats, often referred to as the Felinae or true cats (conical-toothed cats), and the extinct saber-toothed cats in the subfamily Machairodontinae (Turner, 1997). In modern cats, the shape of the skull and mandible enables uniform powerful biting irrespective of body size, whereas in saber-toothed cats, the evolution of shape appears to have been governed by selection for efficient predation, and bite forces were secondary (Christiansen, 2008).

The Panthera lineage split off from the Felinae about 10.8 Mya (Johnson et al., 2006). The earliest leopards, *P.pardus*, are known from Africa from about 2 Mya, and the first leopards appeared in Eurasia about 1 million years later (Werdelin et al., 2010; Werdelin and Lewis, 2005).

1.3.2. Taxonomy

As one of the most widely distributed cats of the world, the leopard shows high polymorphism in morphology (Khorozyan et al., 2006; Meijaard, 2004; Pocock, 1930; Stuart and Stuart, 1991). This has been associated with a wide range of habitat conditions (Gavashelishvili and Lukarevskiy, 2008; Sunquist and Sunquist, 2002) and prey types (Hayward et al., 2006; Norton, 1984). Accordingly, taxonomists have described up to 27 subspecies based on the species' geographic range and phenotypic variation (Ellerman and Morrison-Scott, 1966; Herrington, 1986). Genetic analysis verified nine valid leopard subspecies, eight in Asia and one in Africa (Miththapala et al., 1996; Uphyrkina et al., 2001). Recently, Kitchener et al. (2017) endorsed two east Asian leopard subspecies *P.p.orientalis* and *P.p.japonensis* as synonymous because of the lack of any major barrier to interbreeding, reducing the accepted number of leopard subspecies to eight.

Western Asia and the Caucasus have been known to support seven putative subspecies *(P.p.dathei, P.p.sindica, P.p.saxicolor, P.p.ciscaucasica, P.p.jarvisi, P.p.nimr, P.p.tulliana)* (Herrington, 1986; Zukowsky, 1964). Their geographic extent has been debated (Khorozyan et al., 2006). The west Asian leopards are phylogenetically distinct (Miththapala et al., 1996;

Uphyrkina et al., 2001), supported by craniometric analysis (Meijaard, 2004). It is believed that that no significant geographical barriers are present that would lead to morphogenetic isolation of the subspecies in this region (Uphyrkina et al., 2001), with the exception of the population of the Arabian Peninsula whose isolated position from other west Asian leopards was supported by both genetic and morphometric analysis (Khorozyan et al., 2006; Uphyrkina et al., 2001).

Later, genetic assessment using wild-caught samples supported the presence of a single subspecies in the region, except for western Turkey which needs further assessment (Rozhnov et al., 2011). Additionally, a morphogenetic study on a larger sample of leopards exclusively from Iran supported a monophylum for the region's leopards (Farhadinia et al., 2015a). Kitchener et al. (2017) assumed that *P.p.tulliana*, *P.p.saxicolor* and *P.p.ciscaucasicia* are synonymous, and proposed the application of *P.p.tulliana* as the earliest name for leopards from south west Asia according to the principle of priority belonging to the International Code of Zoological Nomenclature.

1.2.3. Morphometry

Leopards show sexual dimorphism in skull characters (Farhadinia et al., 2014a; Meijaard, 2004; Miththapala, 1992; Pocock, 1930; Stuart and Stuart, 1991). They also have remarkable variation in body size across their global range, ranging between 30 and 90 kg (see Stein and Hayssen, 2013). Persian leopards attain large body mass in west Asian montane landscapes (Dareshuri & Harrington, 1976). Iran's leopards were larger than those of the rest of the species range (Bailey, 1993; Farhadinia et al., 2014a; Stander, 1997; Stein and Hayssen, 2013), with the exception of an Indian sample showing similar descriptive statistics (Athreya and Belsare, 2008). Craniometric investigation has revealed that larger Iranian individuals normally belong to the northern range

(Farhadinia et al., 2014a). Also, adult male Persian leopards were on average 46% heavier than adult females in Iran (Table 1.1; Farhadinia et al., 2014a).

Table 1.1 Body measurement of Persian Leopards in Iran (obtained from Farhadinia et al., 2014a)

		Total Body Length (cm)				**Body Weight (kg)**			
	Sex	**n**	**Mean ± SE**	**Min-Max**	***P***	**n**	**Mean ± SE**	**Min-Max**	***P***
Adult	♂	21	221.1±2.9	200-240	0.034	21	65.8±3.6	40-91	0.009
	♀	11	208.7±5.3	182-228		6	44.8±5.6	26-60	
Sub-adult	♂	4	205.5±10.7	178-230	0.083	3	37.3±5.4	27-45	0.399
	♀	3	177.3±3.5	172-184		3	29.8±5.9	23-41.5	
Cub	♂	0	NA	NA	NA	0	NA	NA	NA
	♀	4	94.5±2.1	55-151		4	8.3±1.4	5.5-12	

1.3.4. Diet

Leopards have the broadest diet among big cats (Sandom et al., 2018), but the commonest prey are medium-sized ungulates within the weight range 10–40 kg with a modal size of around 23 kg (Hayward et al., 2006). In west Asia and the Caucasus, the species is known to mainly prey on bezoar goat *Capra aegagrus*, Urial wild sheep *Ovis orientalis* and wild pig *Sus scrofa* (Farhadinia et al., 2014b; Ghoddousi et al., 2016; Ziaie, 2008), which corresponds well with the high range overlap observed between leopards and bezoar goats throughout Iran (Harrington, 1977; Sanei et al., 2016; Ziaie, 2008). Urial wild sheep has been noted as the main prey in Kopet Dag region, Iran-Turkmenistan borderland (Heptner and Sludskii, 1992), while bezoar goat, wild pig and roe deer *Capreolus capreolus* are considered as key prey species in the Lesser Caucasus

(Khorozyan et al., 2008) together with tur *C.cylindricornis* and red deer *Cervus elaphus* in the Greater Caucasus (Lukarevsky et al., 2004).

1.3.5. Reproduction

In South Africa, leopards appeared to exhibit a birth pulse; most litters are born in the wet season (Balme et al., 2013). The mating season of Persian leopards peaks in mid-winter, with a birth pulse apparently taking place in mid-spring (Farhadinia et al., 2009; Ghoddousi et al., 2008). Males and females associate briefly during mating periods which may last for two to seven days (Bailey, 1993) and just after the short mating period, they separate.

Mean age at first parturition is 46 ± SE 2 months (Balme et al., 2013). After a gestation period of 96 days (ranging from 90 to 106 days), the animal gives birth (Sunquist and Sunquist, 2002), with average litter size 1.9 ± SE 0.1 (Balme et al., 2013). In Iran most litters at age of >6 months are composed of 1-2 cubs (Farhadinia et al., 2009). They reach independence after 19 ± SE 1 months to leave their mother (Balme et al., 2013) and siblings may remain together for several months before separating (Bailey, 1993; Stander et al., 1997). Fattebert et al. (2015) found that sub-adult females stayed in their natal ranges but made exploratory movements elsewhere, whereas sub-adult males had unstable home ranges and explored large areas. In Namibia, two male cubs remained close to their mothers' home range for approximately 8 and 6 months before dispersing from maternal range (Stander et al., 1997). Infanticide can account for almost half of known-cause cub mortality (Balme and Hunter, 2013).

Intraspecific hostility is an important source of mortality among the well-studied high-density leopard populations in South Africa (Balme et al., 2009) where it has been associated with competition over resources including kills (Steyn and Funston, 2006) or territory (Balme and Hunter, 2004). It may also involve cannibalism (Steyn and Funston, 2009) and infanticide (Balme et al., 2009; Balme and Hunter, 2013).

In Africa intraspecific strife among adult and sub-adult leopards is also common, and the predominant documented cause of mortality among female leopards in protected areas of southern Africa (43% of cause-specific records; Swanepoel et al., 2015). The main causes of aggression between female leopards appear to be competition for food resources (Balme and Hunter, 2004) and increased rates of encounter between unfamiliar individuals due to high turnover and thus immigration (Balme et al., 2009). Little has been documented of these phenomena in Asia.

1.3.6. The current status of the Persian leopard in west Asia

After the disappearance of the Asiatic lion *(P. leo persica)* and the Caspian tiger *(P. tigris virgata)* by 1970, the Persian leopard is the largest cat in western Asia, ranging from Afghanistan and Turkmenistan through Iran to Iraq and the Caucasus (Jacobson et al., 2016). Currently, more than 75% of the extant range of *P.p.saxicolor* is within Iran (Jacobson et al., 2016).

Leopards live in a variety of habitats, mainly avoiding deserts, areas with long-duration snow cover, and urban development (Gavashelishvili and Lukarevskiy, 2008). Their habitat in west Asia is predominantly associated with rugged terrain, positively correlated with vegetation productivity (Gavashelishvili and Lukarevskiy, 2008; Zimmermann et al., 2007). Although their remarkable adaptability has contributed to a broad geographic range, some regional populations have declined to critically low levels. In Afghanistan, although the leopard once occurred across vast parts of the country (Habibi, 2003), the species' current situation is uncertain (Stevens et al., 2011). In Turkmenistan, Persian leopards probably have the largest non-Iranian population in the region, mostly around the Kopet Dag Mountains (Atamuradov et al., 1999; Farhadinia et al., 2015b; Kaczensky and Linnell, 2015; Sanei et al., 2013; Ziaie, 2008). The leopard population was steadily declining in Turkmenistan, until first reserves were established along the Kopet Dag Mountains in the 1970s. The total number of leopards in Turkmenistan probably does not exceed 30 to 40 animals (Atamuradov et al., 1999). Likewise, recent monitoring has shown limited presence in the eastern Greater Caucasus and Iori-Ajinour Plateau, but a persistent population in exists in the southern Caucasus (Askerov et al., 2015; Yarovenko and Zazanashvili, 2016), with four putative separate transboundary populations (Askerov et al., 2015). A small number of leopards also persist in the borderland between Iran, Turkey and northern Iraq (Avgan et al., 2016). As a result, the Persian leopard has been listed as *Endangered* by the IUCN since 1996 in recognition of its greatly reduced distribution and population size (Khorozyan, 2008).

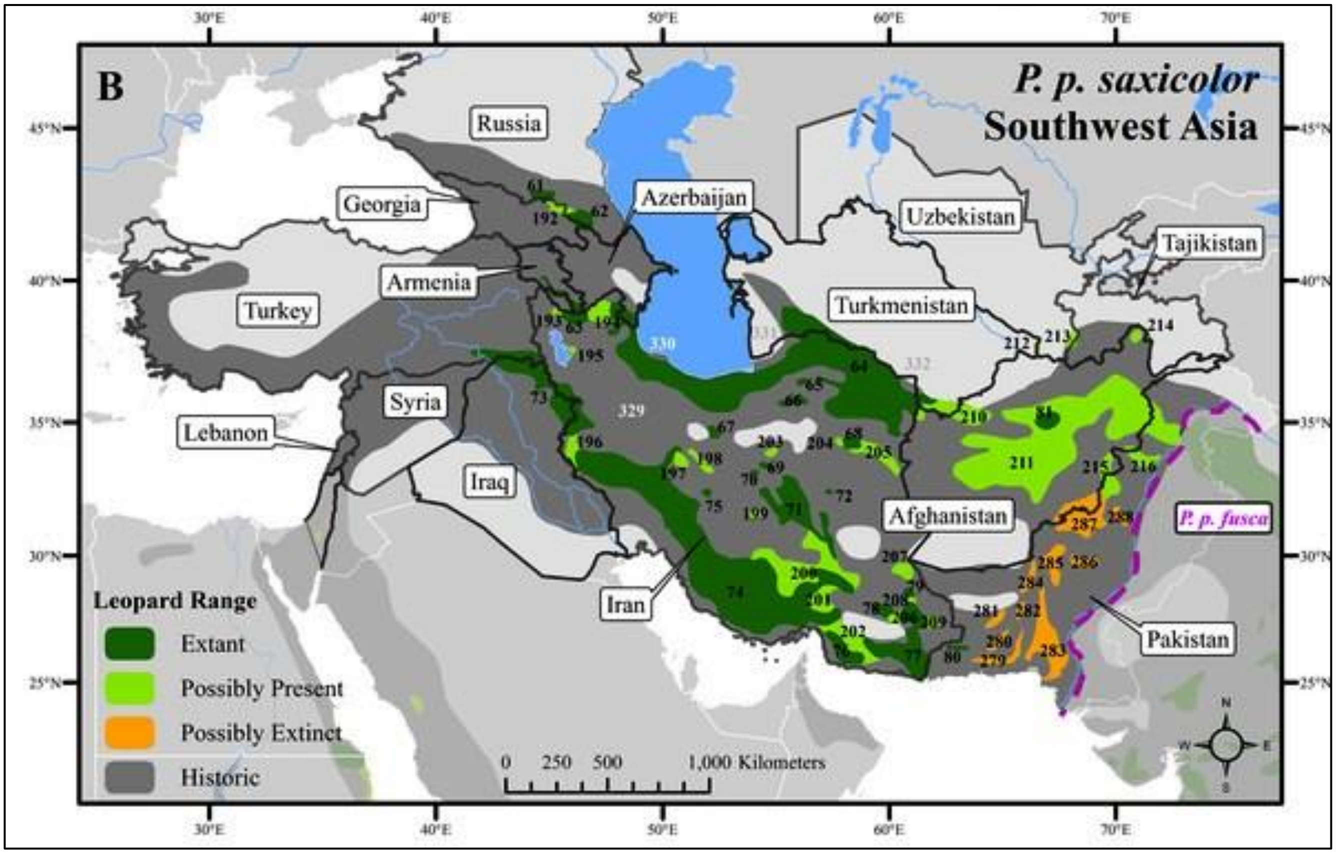

Fig 1.1 Range of Persian leopard in southwestern Asia (Jacobson et al., 2016). Accordingly, possibly present areas are regions where the leopard may possibly occur but recent records are lacking. In contrast, possibly extinct areas are regions where the leopard used to occur but there are no confirmed records in the last 21 years and they are unlikely still present due to habitat loss or other threats.

1.3.7. The Persian leopard in Iran: current status and threats

Except for the desert regions in central and eastern Iran, the leopard historically ranged throughout most of the country (Sanei et al., 2016; Ziaie, 2008). Currently, almost 200,000 km^2, equal to 12% of the country’s area, is modelled as suitable habitat for leopard, but not necessarily occupied by them. Protected areas only cover 58% of the suitable habitats (Ebrahimi et al., 2017).

Iran supports the largest population of Persian leopards, estimated to number between 550-850 animals (Khorozyan, 2008; Kiabi et al., 2002). Density is low, varying between 0.06 and 0.1 individuals/100 km (Kiabi et al., 2002), although they can reach higher densities in well-protected reserves, up-to 2.6 individuals/100 km^2 (Ghoddousi et al., 2010; Hamidi et al., 2014).

Human-mediated mortality is a major threat for the survival of leopards in Iran. They are predominantly targeted intentionally, either as retaliation after stock raiding or occasionally for fur (Sanei et al., 2016). The collapse of the leopard's natural prey due to poaching has led to increased human-leopard conflict in various parts of the country, as leopards are persecuted in response to livestock depredation (Abdoli et al., 2008; Ghadirian and Ghasemi, 2012; Sanei et al., 2016).

The Iranian leopard population is composed of several large discontinuous habitat patches (Ebrahimi et al., 2017; Sanei and Zakaria, 2011). These habitat patches are further threatened by fragmentation (Sanei et al., 2016). It has been predicted that 63% of the current suitable leopard habitats will be lost in the next 63 years, based on different climate change scenarios (Ebrahimi et al., 2017).

1.4. Thesis overview and aims

The Persian leopard population in Iran is the largest population of leopards left in west Asia and the Caucasus. As leopard populations become more fragmented and encroached by humans, understanding their ecological needs will become increasingly necessary for conservation planning. It is hoped that these studies will provide a basis for future research on the applied ecology and biology of leopards in the region as well as providing an evidence base for developing conservation strategies for the long-term survival of the Persian leopards. In this thesis, I explore five key “fundamental” topics (Jacobson et al., 2016) concerning the biology and ecology of leopards, each presented as an independent chapter. I will continue by discussing the implications of “fundamental” chapters to describe the “applied” conservation needs of Persian leopards.

My five “fundamental” objectives are to quantify and explore 1) interaction between large carnivores and local people and how it affects community attitudes towards the species; 2) home range requirements and spatial utilization of leopards; 3) feeding ecology and predation behavior of leopards; 4) population density and composition of leopards; and 5) heterozygosity and spatial genetic partitioning in Iran’s leopards.

The summaries below describe the outline and key questions of each chapter in the thesis.

1.5. Thesis outline and key questions

The current thesis is composed of eight chapters:

1.5.1. Chapter 1: General introduction and thesis overview

In this chapter, I present the background and scope of this thesis. Then, I will proceed to outline an overview of key questions that frame the focus of each research chapter. Having defined questions here, I will describe the theoretical, practical or empirical contexts I use in relevant chapters.

1.5.2. Chapter 2: Study area

I give detailed information about northeastern Iran where my DPhil research was carried out. I also briefly describe the three study areas, with particular attention toward their topography, climate, vegetation, large mammals, and human communities.

1.5.3. Chapter 3: Wolves can suppress goodwill for leopards: Patterns of human-predator coexistence in northeastern Iran

A spatially limited low density of wild ungulates and a high density of livestock, are two characteristics of many Asian mountainous regions (Baskin and Danell, 2003; Mallon and Zhigang, 2009). As a result, conflict with large carnivores is widespread (Dar et al., 2009; Kabir et al., 2013; Suryawanshi et al., 2013). Wild prey depletion and spatiotemporal accessibility of domestic ungulates are two possible drivers of conflict depredation (Babrgir et al., 2017; Jumabay-Uulu et al., 2014; Khorozyan et al., 2015; Shehzad et al., 2015; Zanin et al., 2015). Thus, in areas with a wild prey base and free of domestic animals one could expect low levels of leopard depredation on livestock, and less hostility towards leopards by the local communities. Based on analysis of semi-structured questionnaire surveys of herders, I explore temporal, spatial, and socio-economic factors affecting perceived conflict and outline factors associated with people's attitudes to predators. This chapter has been published as a paper in *Biological Conservation* (213, September 2017, 210-217). Our paper excited a comment in the journal (Ghasemi et al., 2018), leading us to publishing a follow-up article (Farhadinia et al., 2018).

1.5.4. Chapter 4: Anchoring and adjusting amidst humans: Ranging behavior of Persian leopards along the Iran-Turkmenistan borderland

The wide-ranging spatial requirements of apex predators in Asian montane landscapes with high spatiotemporal variability in resources create formidable challenges for conservation managers. Prey availability and environmental productivity are major factors driving predator space use (Loveridge et al., 2009; Nilsen et al., 2005). Additionally, predator movement patterns are also associated with their population density (Balme et al., 2010; Benson et al., 2006; Goodrich et al., 2010). Current conservation planning for landscapes dominated by humans has elements of two main paradigms, the "coexistence" (land sharing) versus the "separation" (land sparing) models (Chapron et al., 2014; Gompper et al., 2015; Johansson et al., 2016; Phalan et al., 2011). The previous limited research effort on Asian mountain-ranging big cats was conducted in a human-dominated landscape, and tended to highlight the importance of land sharing (Johansson et al., 2016). In this chapter, I study the spatial behavioral ecology of leopards using GPS telemetry in a montane national park with staple wild prey and an absence of people to address the conservation issue related to land use management. Accordingly, I pursue the following three objectives: 1) establishing robust estimates of home range, 2) calculating range overlaps, and 3) evaluating the current boundaries of the national park in relation to leopards' space use. This chapter has been published as a paper in *PLoS ONE*.

1.5.5. Chapter 5: Persian leopard predation patterns and kill rates in the Iran–Turkmenistan borderland

The predatory behavior of leopards has been investigated in different habitat types for nearly a half-century. The components of predation such as kill rate, prey composition and how they vary are helpful for understanding basic ecology and informing effective management (Hayward et al., 2007; Wegge et al., 2009). Problems with estimating components of predation in previous studies include short tracking sessions (19 days; Odden and Wegge, 2009), inadequate sampling of kill sites (Martins et al., 2011), delays in identifying kill sites due to time lapsing before downloading data (Stein et al., 2015), and failure to verify kill sites identified from clusters of movement fixes (Rozhnov et al., 2015), resulting in a wide range of variation in estimates of leopards' kill rates. I use GPS telemetry as the currently recommended method for big cat predation studies (Elbroch et al., 2017; Knopff et al., 2009).

I address four objectives concerning the foraging behavior of Persian leopards: 1) leopard prey choice and how it is affected by spatiotemporal factors; 2) kill rate and consumption rates (prey per individual per month); 3) foraging behavior and how feeding time is affected by prey body mass and kill location; and 4) predation pattern and its associated correlates (prey body mass, season and location). This chapter has been published as a paper in the *Journal of Mammalogy*.

1.5.6. Chapter 6: A paradox of local abundance amidst regional rarity: the value of montane refugia for Persian leopard conservation

Leopards have a great variation in density estimates across their global range. Estimates of leopard population densities vary 150-fold from 0 ~ 0.1 to 14.9 individuals per 100 km^2 (Harihar et al., 2009; Jiang et al., 2015). Surveys from the protected areas of west Asia and the Caucasus region have reported densities at the lowest known extreme for the species, fewer than 0.5 individuals/100 km^2 (Khorozyan et al., 2008; Spalton et al., 2006). In this chapter, I employ a spatially-explicit capture recapture method (Efford, 2004; Royle et al., 2009) by means of motion-detector camera traps in three national parks in northeastern Iran to answer two questions: 1) population density and composition of leopards; and 2) the effect of camera trapping at water resources during summer on estimates of population density and composition.

1.5.7. Chapter 7: Genetic variation and population structuring of Persian leopards

Leopards show higher genetic diversity than many other big cats (Eizirik et al., 2001; Luo et al., 2004; Uphyrkina et al., 2001). Nonetheless, their heterozygosity is not spatially homogenous across their global distribution (Uphyrkina et al., 2001), varying 2.5-fold between the most variable populations in Africa and India (Dutta et al., 2012; Ropiquet et al., 2015) to least variable populations observed in the Far East of Asia and the Arabian Peninsula (Perez et al., 2006; Uphyrkina et al., 2002). Unlike taxonomy and phylogeography which are moderately well-studied (Farhadinia et al., 2015a; Khorozyan et al., 2006; Kitchener et al., 2017; Rozhnov et al., 2011; Uphyrkina et al., 2001); our understanding of spatial population structuring and the genetic variability of west Asian leopards is poor.

In this chapter, I use multiple nuclear and mitochondrial genetic markers to describe patterns of population partitioning and genetic variability in the west Asian leopard’s main population hotspot, i.e. Iran. In west Asia, most of the leopard distribution is non-continuous (Jacobson et al., 2016), generating a hypothesis that limited gene flow and genetic connectivity have shaped spatial genetic structuring in leopards with reduced genetic variability. Equally important, by applying mitochondrial DNA sequences and microsatellite loci size variations, I evaluate the genetic status of Persian leopards and compared it with other leopard subspecies.

1.5.8. Chapter 8: General discussion and conservation implications

In this chapter, I synthesize the key findings from chapters 3 to 7. I then consider the implications for conservation. In particular, I highlight the management implications of ecological studies I presented in chapters 3 to 7. Finally, I describe important areas for future research.

Appendices

During the course of the work for this DPhil, I contributed to a number of academic papers in peer-reviewed journals related to the aims of this thesis and my career as a conservation biologist. I include some of the most relevant papers as appendices.

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Chapter 2:

Study Area

Study area

In this chapter I briefly outline the geographic location and topographic features of northeastern Iran and the Kopet Dag Ecoregion. I go on to report climate, vegetation and the main mammalian species related to my thesis. Finally, I describe my three study areas.

2.1. Northeastern Iran

Historically, northeastern Iran was equivalent to the former Khorasan Province, which was recently split into three smaller provinces of North Khorasan (28,434 km^2), Razavi Khorasan (118,854 km^2) and South Khorasan (151,193 km^2). The Kopet Dag Mountains are located in the northern parts of northeastern Iran and are composed of two parallel mountainous chains, i.e. the northern chain (Kopet Dag and Hezar Masjed mountains) and the southern chain (Binaloud, Aladagh and Golestan mountains) (Fig 2.1; Afshar-Harb, 1994). Their western extension joins the Alborz Mountains along the deforming Alpine - Himalayan orogenic belt in the south Caspian basin (Mangino and Priestley, 1998).

Fig 2.1 Map of northeastern Iran and the two main mountainous chains along border with Turkmenistan (www.flickr.com)

Generally, both mountain chains, along with their Turkmen counterparts are referred to as the Kopet Dag. With an area of almost 165,000 km^2, Kopet Dag lies between 34°20' and 39°13' N latitude and 55°05' and 61°20' E longitude. According to Memariani et al. (2016), "It is delimited sharply in the north and northwest by the Karakum desert; in the east by the Iran-Turkmenistan border along the Tajan River and western Badghyz and partly by the Iran-Afghanistan border along the Harir-Rud River and the western extension of the Paropamisus Range; and in the south and southwest by the central Iranian deserts. To the west the area is connected partly to the Gorgan plain and Turkman-Sahra salt desert and partly to the eastern extension of the Alborz mountain range and the Hyrcanian forests of the Euro-Siberian region (Fig 2.2)".

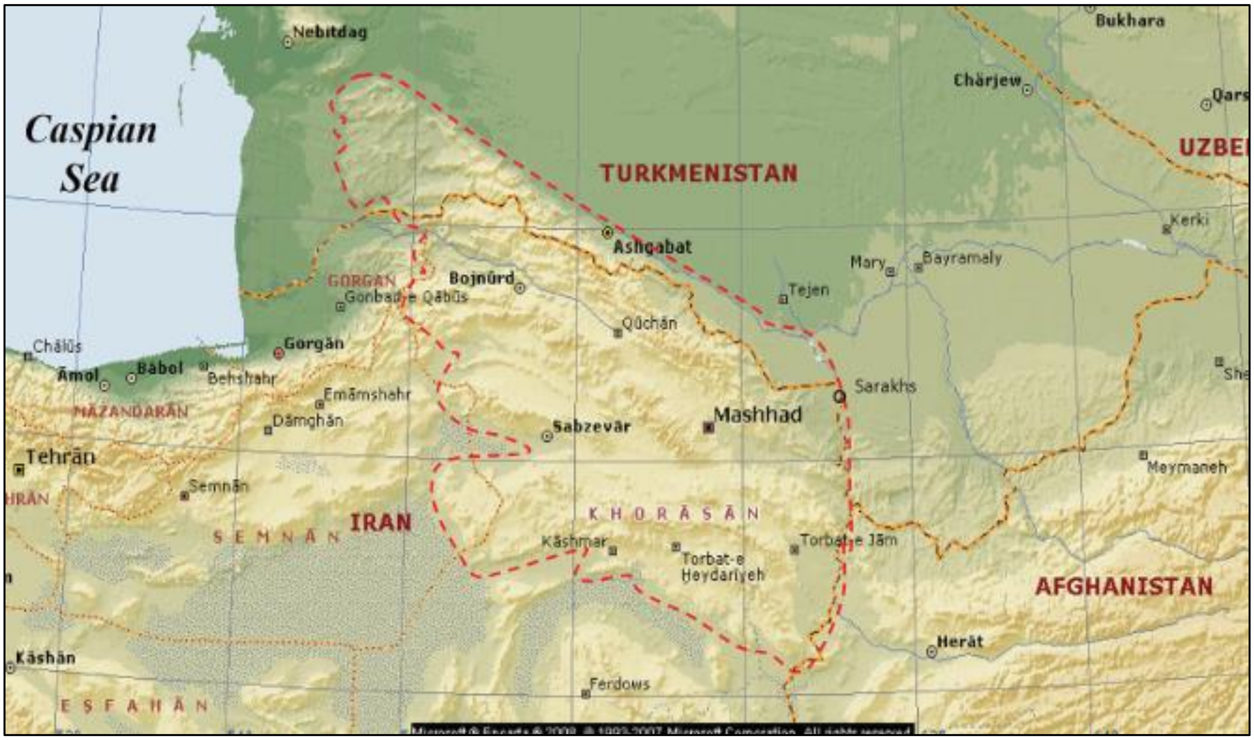

Fig 2.2 Geographical delimitation of Kopet Dag region, delineated by the red dotted line (map obtained from Memariani et al., 2016)

In Turkmenistan, the Kopet Dag mountains and Karakum desert are separated only by a narrow (10 to 20 km) belt of the arid foothills, creating a biogeographic border between the Mediterranean-type mountains of Kopet Dag and the lowland continental desert of the Karakum (Atamuradov et al., 1999). The Kopet Dag sedimentary basin formed after the Middle Triassic orogeny in northeastern Iran as a sink along the major faults aligned approximately NW-SE (Shafieeardestani et al., 2013). The current DPhil study was carried out in multiple study areas along both the Kopet Dag and Aladagh Mountains in northeastern Iran.

2.2. The Kopet Dag Ecoregion

Globally, there are 34 biodiversity hotspots. Iran hosts two of them, i.e. Caucasus and Iran-Anatolian (Mittermeier et al., 2004). The latter is a topographically complex and extensive system of mountains and closed basins that form a natural barrier between the ecosystems and indigenous cultures of the Mediterranean Basin and the dry plateaus of western Asia. For many centuries, the Silk Road crossed east to west through this hotspot, culturally connecting the two regions. The hotspot covers 899,773 km^2, including major parts of central and eastern Turkey, a small part of southern Georgia, the Nakhchivan Province of Azerbaijan, much of Armenia, northeastern Iraq, northern and western Iran, and the northern Kopet Dag range in Turkmenistan (CEPF, 2016). Nested within the Irano-Anatolian biodiversity hotspot, Kopet Dag is one of the global 200 known ecoregions and is shared between Iran and Turkmenistan (Olson and Dinerstein, 1998).

2.3. Climate

According to Memariani et al. (2016), the climate of Kopet Dag is distinctly continental, due to the distance from the oceans, with a mean annual precipitation usually of 175–300 mm in the plains and foothills and 300–380 mm in the high mountains (Fig 2.3). The rainfall distribution is uneven throughout the year, mostly occurring in late autumn, winter and early spring (October to May). A prolonged summer drought (June to September) is common. The climate of the area is distinctly drier than in the neighbouring mountainous areas.

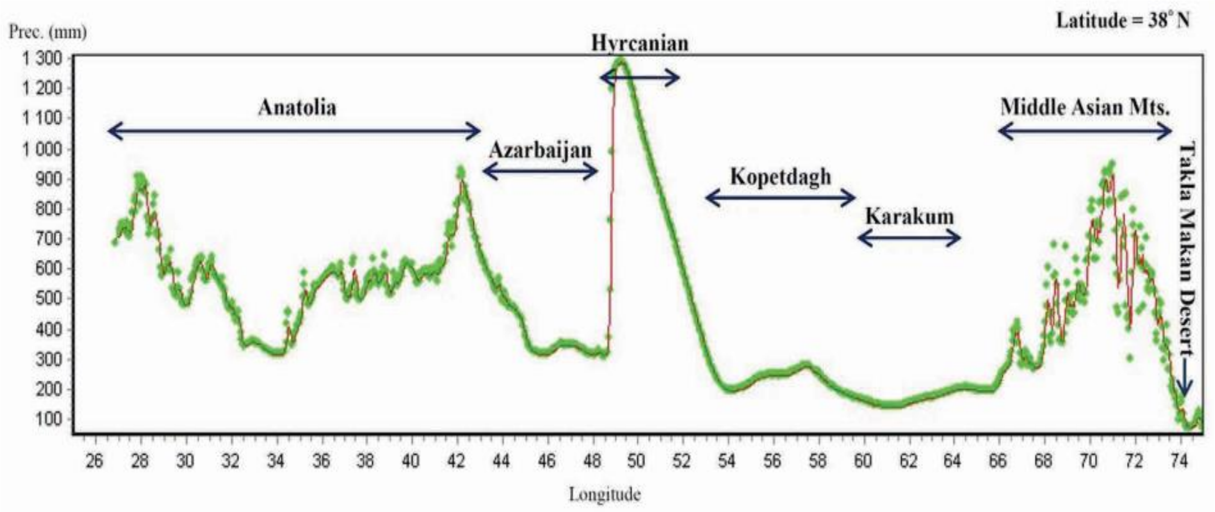

Fig 2.3 Transect of mean annual precipitation (mm) from Anatolia (W Irano-Turanian) to the Middle Asian Mountains (E Irano-Turanian) along 38 N latitude, with the position of the Kopet Dag Mountains (map obtained from Memariani et al., 2016)

2.4. Vegetation

The Khorasan-Kopet Dag floristic province is a transition zone connecting different provinces of the Irano-Turanian region and the Hyrcanian montane forests of the Euro-Siberian region. A total of 2576 species/infraspecific taxa belonging to 702 genera and 112 families of vascular plants have been reported from the Iranian side, including 356 species (13.8%) endemic to the region (Memariani et al., 2016). In Turkmenistan, up-to 18% of endemic vascular plant species are believed to have originated from the Ancient Mediterranean stock common for all floras of Central Asia (Atamuradov et al., 1999). The region's fauna and flora include a combination of western (mostly Mediterranean) and eastern (Turaninan) elements, as well as local autochthonous endemic species (Fet, 1994).

2.5. Leopards and their prey

The Kopet Dag mountains are well-known for Persian leopards (Atamuradov et al., 1999; Farhadinia et al., 2015; Sanei et al., 2013; Ziaie, 2008). On the Turkmen side, the leopard population was steadily declining, until the establishment of the Kopet Dag Reserve in 1976, and the Syunt-Khasardagh Reserve in 1979. Since then, regular observations of leopards have been made during the 1980s in central and western Kopet Dag (Atamuradov et al., 1999). In Iran, the leopard has always been frequently sighted within several reserves established since the 1960s (Farhadinia et al., 2014; Hamidi et al., 2014; Sanei et al., 2013; Ziaie, 2008), but there are no population data on the state of the leopards in northeastern Iran. The total number of leopards in Turkmenistan probably does not exceed 30 to 40 animals (Atamuradov et al., 1999).

Besides the Persian leopard, Kopet Dag is also well-known for urial sheep *Ovis orientalis* (Atamuradov et al., 1999; Harrington, 1977; Jamshid, 1975; Valdez et al., 1977; Ziaie, 2008). By the end of the 1990s, it was estimated that ca. 7,000 urials existed in Turkmen Kopet Dag (Atamuradov et al., 1999). Although no official population record for urial in Iran is available, based on unpublished data from the Iranian Department of Environment obtained during its annual total counts, at least 7,290 urials occured within protected areas by mid 2010s (Tandoureh <2000, Salouk ~ 1500, Sarigol ~ 1200, Sarani ~ 350, Jangal Khajeh ~ 1050, Heydari ~ 600, Ghorkhod ~ 200, Dorbadam ~150, Gharchegheh ~ 140).

It is also guessed that at least 3000 bezoar goats *Capra aegagrus* live in a number of reserves on the Iranian side, mostly in Tandoureh and Ors-Sistan. By the end of the 1990s, the bezoar goat population in Turkmenistan was estimated to be around 2,000 (Atamuradov et al., 1999).

2.5. Study areas

Located in eastern extreme of the Irano-Anatolian Biodiversity Hotspot, the Kopet Dag and Aladagh Mountains in northeastern Iran host a number of montane reserves, including Tandoureh, Salouk and Sarigol which hosted my study (E57°15’ to E59°15’, N36° 20’ to N37°20’; Fig 2.4 & Table 2.1).

Located in two provinces of North Khorasan and Khorasan Razavi, these three areas in total cover almost 930 km^2 of mountainous landscapes with an altitude range of 1000 to over 3000 meter a.s.l., steep cliffs and deep valleys. Generally, the mean annual precipitation and temperature are 200 to 300 mm and ca. $15^{o}C$, respectively, leading to the development of a temperate semi-arid climate (Darvishsefat, 2006).

Table 2.1 Summary of Persian leopard wide field survey effort for northeastern Iran. NP = National Park and PA = Protected Area.

Study site	Protection Category*	Year of Establishment	Area (km^2)
Sarigol	NP & PA	1974	280
Salouk	NP & PA	1973	200
Tandoureh	NP & PA	1968	450

The vegetation is generally dominated by scrub species, particularly *Astragalus* spp. and *Artemisia sieberi* forms bush-steppe habitat in most of the areas, with pockets of juniper *Juniperus* spp. and barberry *Berberis* spp. (Darvishsefat, 2006). Potential ungulate prey in the leopard diet includes urial, bezoar goat, and wild pig *Sus scrofa*. These areas also support a diverse range of carnivores, including grey wolf *Canis lupus*, striped hyena *Hyeaena hyeaena*, African wild cat *Felis lybica*, and Pallas's cat *Otocolobus manul* (Ziaie, 2008).

My study areas are divided between two different categories: National Park and Protected Area (hereafter NP and PA). NPs have a higher level of law enforcement and livestock grazing is not permitted. PAs are still protected, but with lower a level of anti-poaching efforts. Furthermore, a number of nomadic pastoralists’ herds are permitted to graze in the PAs during summer (May-August) each year. Moreover, local people living in surrounding villages keep domestic animals, mainly sheep *O. aries* and goat *C. hircus*.

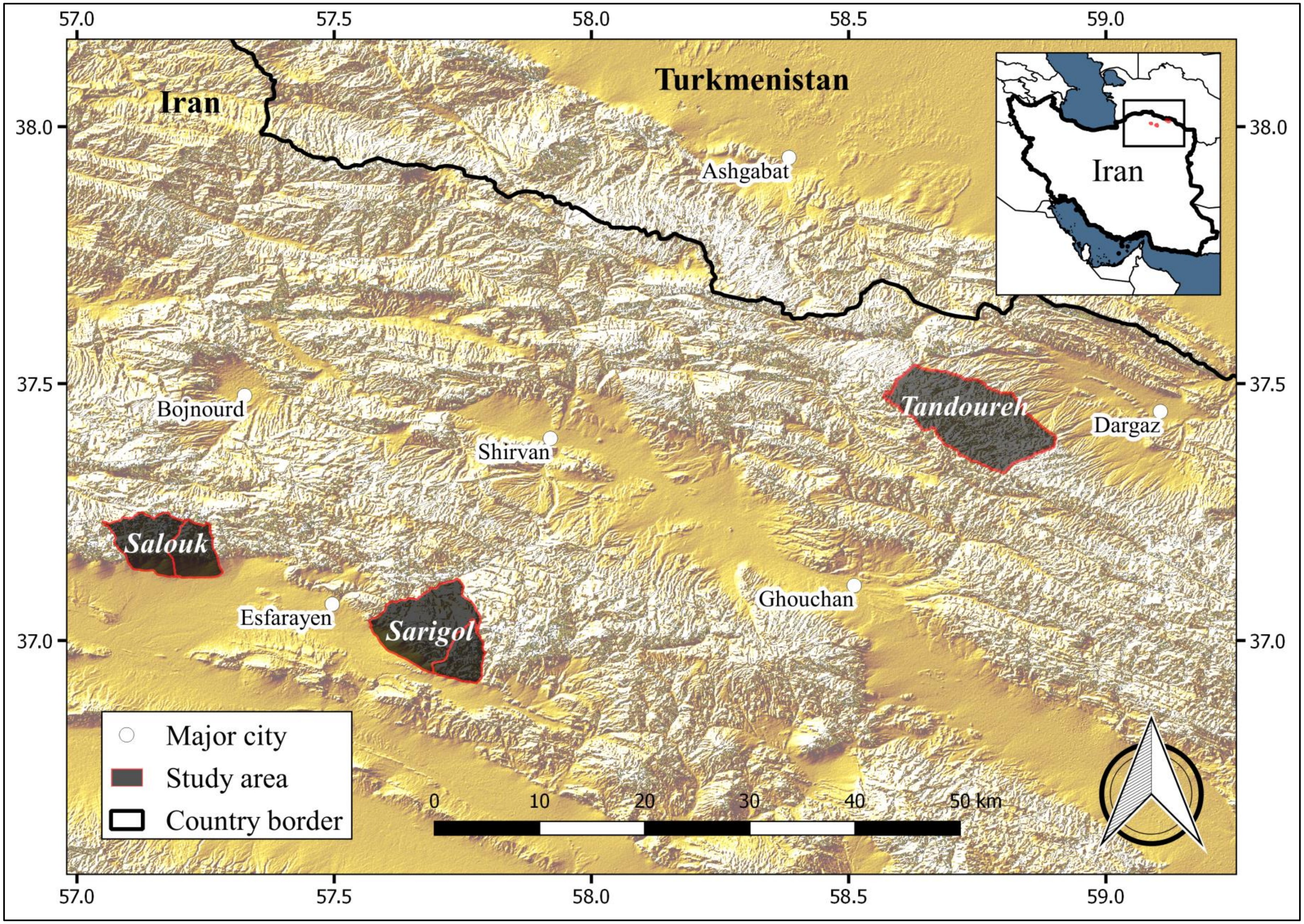

Fig 2.4 Spatial configuration of the three study areas in northeastern Iran.

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Chapter 3:

Wolves can suppress goodwill for leopards: patterns of human-predator coexistence in northeastern Iran

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Wolves can suppress goodwill for leopards: patterns of human-predator coexistence in northeastern Iran

3.1. Abstract

Mammalian carnivores often cause problems for people by preying on domestic stock. Exploring the factors that affect people's attitudes to predators, in particular the circumstances when some degree of loss is tolerated, is needed for developing predator management plans. When more than one species of predator is involved, there may be unexpected interactive effects in shaping people's tolerance. We addressed this hypothesis in a west Asian multi-predator montane landscape with high density of both wild ungulates and livestock harboring two large predators, Persian leopard and grey wolf. A semi-structured questionnaire survey of herders residing around Tandoureh, Salouk and Sarigol National Parks, northeastern Iran was carried out. The perceived role of leopards in depredation was negligible compared with that of wolves which were reported to be more frequent stock raiders and responsible for an average of 5.7 times more annual losses per herd by than were leopards. Non-predatory causes of mortality, particularly diseases, were clearly the major threat to livestock. Interviewed herders showed different attitudes toward each predator. Regardless of any recent occurrence of stock raiding by wolves, they were predominantly considered negatively. Although people showed mainly positive attitudes toward leopards, respondents who reported more wolf attacks tended to have more negative attitudes toward leopards. Hence, in multiple predator ecosystems, peoples' attitudes toward each species may be affected by the perceived activity of other predators. Often neglected in conservation programs, this phenomenon is clearly important in sustaining people tolerance particularly if endangered large predators are involved.

Keywords: Persian leopard, predator guild, conflict, attitude, depredation, Iran.

3.2. Introduction

The arid montane landscapes of west and central Asia host a low density of wild ungulates, mostly confined to protected areas (Baskin and Danell, 2003). They are also densely occupied by increasing numbers of pastoral herds of small stock (Mallon and Zhigang, 2009). It is estimated that west and central Asia harbor more small-bodied livestock than North and South America combined (Thornton, 2010). As a result, competition over limited resources between wild and domestic ungulates is inevitable (Namgail et al., 2007) and conflict with large carnivores is widespread (Dar et al., 2009; Kabir et al., 2013; Suryawanshi et al., 2013).

A number of large carnivores share the montane areas of west and central Asia with humans, with the leopard *Panthera pardus* and the grey wolf *Canis lupus*, generally causing the greatest level of conflict. They are subject to different patterns of tolerance and attitudes by human communities across their very large geographic ranges (Kansky et al., 2014). The co-existence of leopards with humans is often characterised by moderate to severe levels of conflict (Dar et al., 2009; Kabir et al., 2013; Khorozyan et al., 2015; Shehzad et al., 2015). The conflict is unsurprisingly often linked to the extent to which they kill domestic stock (Babrgir et al., 2017; Shehzad et al., 2015) or domesticated carnivores, particularly dogs (Farhadinia et al., 2015; Ghoddousi et al., 2016). The grey wolf is also generally considered as a nuisance due to its consumption of livestock (Kikvidze and Tevzadze, 2015; Suryawanshi et al., 2013), even in areas with abundant multiple wild prey species (Hosseini-Zavarei et al., 2013).

People’s attitudes toward predators are not shaped solely by perceived economic loss, however, and may be influenced by a wide range of socioeconomic factors (Babrgir et al., 2017; Dar et al., 2009; Hosseini-Zavarei et al., 2013). Age, gender, and education (Suryawanshi et al., 2014), risk to human life (Behdarvand and Kaboli, 2015) and beliefs about predator behaviour (Kikvidze and Tevzadze, 2015) can play a role.

Spatiotemporal accessibility of domestic ungulates is known to be the driving factor for large felid depredation (Jumabay-Uulu et al., 2014; Miller et al., 2015; Zanin et al., 2015). Thus, even in areas with high wild prey availability but with easily accessible stock resources, large felids regularly depredate on livestock (Khorozyan et al., 2015; Suryawanshi et al., 2013). Furthermore, wild prey depletion is an additive determinant of livestock depredation by large cats in montane landscapes (Babrgir et al., 2017; Dar et al., 2009; Sharma et al., 2015; Shehzad et al., 2015). Considering these drivers of conflict, we might expect low levels of leopard depredation on livestock, and less hostility towards leopards by the local communities where there is a stable prey base, good law enforcement and where livestock are excluded.

In this paper, we explored patterns of coexistence between humans and large carnivores across a sample of well-protected semi-arid montane reserves in northeastern Iran. We investigated perceived carnivore-caused mortality using the herders' records (i.e. memoirs and impressions) rather than actual loss, because the perceived rather than actual level of depredation often drives powerful negative perceptions to predators (Mishra, 1997).

We investigated temporal, spatial, and socio-economic factors affecting perceived conflict with large carnivores across some key leopard reserves in northeastern Iran. We documented perceived spatiotemporal variation in livestock loss due to wild predators and other causes, by season and by locality. We expected that losses to predators would be considerably exceeded by those to other causes, and we expected that diligent husbandry would reduce depredation. Furthermore, we hypothesized that people's attitudes toward larger carnivores are mainly driven by socio-economic factors. We anticipated that our research would clarify the dynamics of human-carnivore coexistence in a poorly studied ecosystem, as well as assist to design mitigation activities to reduce conflict and inform policy for the conservation of the endangered Persian leopard *P.p.saxicolor.*

3.3. Materials and Methods

3.3.1. Study Area

The Kopet Dag and Aladagh Mountains in northeastern Iran host a number of montane reserves, including Tandoureh National Park and Protected Area, Salouk National Park and Protected Area and Sarigol National Park and Protected Area, lying at the eastern extreme of the Irano-Anatolian Biodiversity Hotspot (E57°15’ to E59°15’, N36° 20’ to N37°20’; Fig 3.1 & Table 3.1). They are a total of almost 930 km^2 of very rugged mountainous landscapes of steep cliffs and deep valleys at altitudes of 1000 to over 3000 meter a.s.l.. Mean annual precipitation and temperature are 200 to 300 mm and around 15^oC, respectively, leading to a temperate semi-arid climate (Darvishsefat, 2006).

The vegetation is generally dominated by scrub species, particularly *Astragalus* spp. and *Artemisia sieberi*, forming a bush-steppe habitat in most areas, with pockets of juniper *Juniperus* spp. and barberry *Berberis* spp. (Darvishsefat, 2006). Potential ungulate prey for leopards include urial *Ovis orientalis,* bezoar goat *Capra aegagrus,* and wild pig *Sus scrofa*. These areas also support a diverse carnivore community, including leopard, grey wolf, striped hyena *Hyaena hyaena*, Eurasian lynx *Lynx lynx,* African wild cat *Felis lybica,* and Pallas's cat *Otocolobus manul* (Ziaie, 2008).

Our three study sites encompass areas of National Park and non-National Park (hereafter NP and non-NP). NPs experience greater law enforcement, and livestock grazing is completely banned. Non-NPs designated in Iran as Wildlife Refuge or Protected Area, have lower levels of protection, and enjoy less intense anti-poaching efforts. Furthermore, nomadic pastoralists are permitted to graze their herds in non-NPs during summer (May-August). Herds are comprised largely of sheep *O. aries* (84% ± 2) with goats *C. hircus*. Livestock grazes in seasonal pastures in wilderness areas for most of the year, but they are herded closer to villages during winter, where the main food stock is often the stubble of crops.

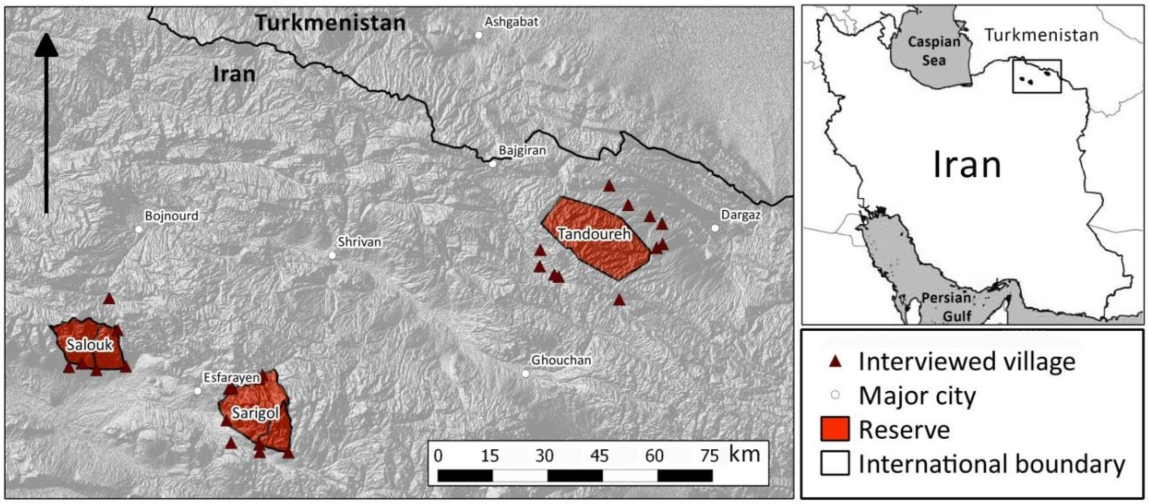

Fig 3.1 Location of investigated villages (triangles) around three study sites in northeastern Iran.

3.3.2. Sampling design

From August 2013 to September 2014, we conducted a semi-structured questionnaire survey with selective open-ended questions, to obtain data on people's attitudes, perceptions and interaction with large carnivores (i.e. Persian leopard and grey wolf). Closed-format questions reportedly result in less uncertainty than open-ended ones, for both the respondent and the researcher (White et al., 2005).

We focused on villages located on the borders of the three study sites and/or their associated herds of livestock spending part of each year within the reserve's pastures (n=29). Inside each village, several households usually merge their herds of domestic stock to create a single large herd, always accompanied by at least one shepherd and several dogs. We targeted these aggregated herds from each village and interviewed the shepherd assigned to each.

We interviewed shepherds from 91 herds (from a total of 100 herds; Table 3.1), representing 302 households living around three reserves, i.e. Tandoureh, Salouk and Sarigol. Each herder was interviewed between 1 and 3 times. However, we used responses only from those respondents who were interviewed more than once during the survey year. This provided data on seasonal patterns of loss. Accordingly, 72 herders' data were entered into the analysis, representing 79.1% of our initial target population. Each herder was interviewed on average 2.3 (SE 0.1) times, representing 269 households (3.7 ± 0.3 households per each herd). Except for the villages of Noushirvan (Sarigol), Haji Ghelichkhan and Doroungar (Tandoureh), we interviewed herders living in all surrounding villages around three reserves, totaling 26 (Fig 3.1 & Table 3.1).

Table 3.1 Details of interviewed herders around multiple reserves in northeastern Iran

	Sarigol	**Salouk**	**Tandoureh**	**Total**
No. villages	9	6	12	26
No. herds	18	22	60	100
No. interviewed herders	12	16	44	72
Percentage of sampling	66.7	72.7	73.3	72.0
Total livestock number of interviewed herds	5,500	10,290	18,229	34,019
Mean herd size (SE)	458.3 (39.3)	643.1 (63.7)	414.3 (47.8)	472.5 (34.8)

3.3.3. Interview surveys

The questionnaire was tested by piloting in a different area with similar herding practices to highlight potential ambiguities (White et al., 2005). Accordingly, each team member was asked to conduct a questionare survey with at least 15 herders for the purpose of assessing human-carnviores interaction. All team members received training based on guidelines for in-person interview-based surveys developed from similar studies (Hosseini-Zavarei et al., 2013; e.g. Majić and Bath, 2010; Zimmermann et al., 2005).

Only one person associated with each herd was interviewed (normally the only person accompanying the herd in the pasture). Before interviews, respondents were asked for consent following an explanation of the research objectives and assurance that all personal information would remain strictly confidential. We conducted unstructured interviews in a friendly environment in order to gain the trust of interviewees and to make the interviews less biased. We evaluated respondents' ability to identify carnivores by showing them photographs of different species known from the area (leopard, wolf, striped hyena and Eurasian lynx). Each interview was limited to 20-30 minutes; animals grazing away from the shepherd made him anxious because unattended herds can be susceptible to attacks.

The questionnaire collected the following information: (1) socio-economic variables (respondents' age, education, as well as the types, numbers, and insurance status of livestock, number of dogs as well as shepherds), (2) details on herders' encounters with leopards in the wild within past five years; (3) experiences of livestock loss due to predation or non-predation causes during the past 3-6 months; and (4) herders' attitudes towards leopards and wolves. There was no active compensation program running during our survey period. Responses were not therefore biased upwards for this reason. Also, we randomly cross-checked responses with other families owning the same herd to verify loss claims.

We asked interviewees about the prevailing market value (at the time of the interview) of each type of livestock (sheep and goat) and then calculated weighted arithmetic mean market price for each herd, based on the proportion of sheep and goat in his herd as well as their associated price. Independently, we also obtained market value from local slaughterhouse to verify the people's perceived value of each livestock. We collected details on recent experiences of carnivore predation on livestock, including season, time, location, number of detected predators (in case the carnivore has been sighted) and number of killed domestic animals. The numbers for losses are as perceived by the respondents, as are the causes of loss. So when phrases like 'wolf losses' are used in the results this is shorthand for perceived losses as reported by the respondents.

We also collected detailed information about non-predatory causes of loss, such as number and timing of mortalities as well as their causes. We accepted disease as the cause of mortality where diagnosis was made by a local vet; otherwise we recorded putative cases as unknown. In order to obtain fine-scale data on losses, we tried to approach the herders in different seasons to ask them for their recent mortalities. Finally, we asked herders to rate their attitude towards leopards and wolves on a Likert scale from 'strongly like=0' to 'strongly dislike=4'.

3.3.4. Statistical analysis

Co-linearity between predictor variables was assumed to occur when r was greater than 0.7 using Pearson product moment correlation analysis. Accordingly, number of households owning each herd was excluded, because it was correlated with herd size.

We used three proxies for efficiency of herding practices: herd size, the number of dogs with the herd and each herd's reported loss due to diseases. To illuminate underlying factors affecting losses to predators, we fitted generalized linear mixed models (GLMM) with a Poisson error distribution. The numeric response variables included number of livestock reported to be depredated by wolf or leopard. Since all herds are generally accompanied by one herder, this was not a useful predictor and was excluded. We used reserve identity as a fixed blocking factor and village identity as a random effect. For models which included a season effect, each respondent contributed a response for multiple seasons; therefore the respondent ID was also included as a random effect, nested in village.

We also built a GLMM predicting the numbers reported lost to each predator (wolf and leopard) to explore spatiotemporal variation in livestock loss. Season (4 levels) and reserve (3 categories) were entered as categorical fixed predictors, with similar random effects as previous models. Again, a GLMM with Poisson error distribution was developed. The lme4 library (Bates et al., 2016) was used to fit all these models with the default Laplace Approximation for estimating maximum likelihood.

Herding characteristics (herder age, current price and number of dogs) as well as frequency of livestock loss to wolf, leopard and diseases were then considered as variables affecting the respective people's attitude toward each predator (as measured on a Likert scale).

Each herd was composed of both domestic sheep and goat which have different market prices, and sometimes respondents were uncertain of the exact species lost. We assumed that stock losses were proportional to their relative abundance in the stock population, and we calculated a weighted arithmetic mean for each respondent, based on proportion of sheep and goat in his herd to which we applied the respective associated price.

To construct models predicting Likert scale ordinal responses, we fitted a cumulative link mixed model with the clmm function in R package ‘ordinal’ (Christensen, 2015). All numeric predictor variables were standardised to z scores.

An information theoretic or ‘IT’ approach was used for model selection. This enables examination of several competing models or hypotheses simultaneously to identify the best set of models (Grueber et al., 2011) via information criteria such as Akaike’s information criterion (Burnham and Anderson, 2002), after correction for small sample size (AICc). Convergence problems for models predicting people attitudes toward leopards using ‘clmm’ led us to use the closely related ‘clmm2’ function in package ordinal. We permitted our GLMM models to have a maximum of four and three parameters for each model fitting to people’s attitude toward wolf and leopards, respectively in order to enable proper model convergence. The categorical blocking effect reserve identity was included in all models as a design feature.

We used package *MuMIn* (Bartoń, 2009), to calculate the model weights. The proponents of this methodology recommend that these weights are best interpreted as the probability for a model that it is the best model for the observed data, given the set of candidate models (Bolker, 2008). We chose not to interpret model-averaged coefficients for predictor variables in order to determine predictor importance, as their interpretation may be problematical when there is multicollinearity among predictor variables, which is unavoidable in observational studies (see Cade, 2015). We conducted all analyses in the *R* environment for statistical computing (R Development Core Team, 2013).

3.4. Results

3.4.1. Interviewee characteristics

Respondents were on average 43.6 (SE 1.3, range 21 to 70, median 43) years old and relied primarily on stock breeding as well as farming. Our survey covered herders with a total of 34,019 domestic ungulates grazing within the study area. Average herd size was 472.5 animals (SE 34.6). Herd size differed among reserves ($F_{2, 69}$= 3.874, P=0.025), being largest in Salouk (643.1 ± SE 54.3). Each herd was accompanied by a shepherd as well as an average of 3.6 (SE 0.3) herding dogs, and there was no evidence that number of dogs per herd varied among sites ($F_{2, 68}$= 0.796, P=0.45).

Only 16.9% of interviewed herders had complete or partial insurance coverage for their livestock. The reasons given for this by interviewees were: ignorance i.e. they lacked awareness of the key authorities involved, lack of financial resources to buy it, and dissatisfaction with the insurance process. This last reason is particularly relevant for livestock loss due to predators, because compensation is conditional on recovering the tagged ear of the killed animal, which is problematic in cases of predation when the carcasses is not located.

3.4.2. Knowledge, attitude and perception

Almost all respondents were able to identify wolves and leopards based on photographs; lynx were relatively unfamiliar (Fig 3.2). Furthermore, most herders had reported seeing individuals of leopard, hyena and wolf over their lifetime; the latter was seen by all interviewees, while about 80% had seen hyena and leopard (Fig 3.2). Only the responses of those who recognized leopard and wolf were used in the analyses regarding this species. Twenty two percent of interviewees (n=16) reported leopard attacks on humans for the preceding five years, while only four respondents were attacked themselves by leopards. All attacks happened when herders tried to push away leopards from their stock. In contrast, only five herders reported wolf attacks on humans.

62.5% of interviewed herders (n=45) reported an encounter with leopards during the past five years, resulting in 68 sightings within or around the three reserves. All herders had made recent (i.e. within 5 years) sighting of wolves. Almost all herders (98.4%) perceived the grey wolf to be the main predator of their domestic stock. A Wilcoxon Signed-Ranks Test indicated that herders' attitude toward the predators was significantly different, i.e. attitude toward the wolves was strongly negative compared to the leopards where attitudes tended to be positive (Z=124.5, $P<0.001$; Fig 3.3).

3.4.3. Spatiotemporal patterns of livestock loss

The questionnaires produced information about all three large carnivores taking livestock, although the striped hyena was reportedly seen only once killing a sheep, in Tandoureh. 29 herders (40.3% of total interviewees) experienced stock loss perceived to be due to leopards during the previous year, whereas most of the interviewees (93.1%) reported experiencing wolf attacks during the previous year. Herders reported that they lost fewer animals per herd to leopards (mean 0.9 ± SE 0.2) compared to wolves (mean 5.1 ± SE 0.6; ranging 1 to 10), (Paired t-test=-6.15, df = 71, $P<0.001$), yielding a ratio of 1:5.7 annual livestock loss to leopards and wolves, respectively (Fig 3.4). Stock loss due to leopards and wolves were reported to be 0.4 (SE 0.1) and 1.5 (SE 0.2) animals, respectively between August 2013 to September 2014. There was no evidence of correlation between the number of livestock lost to wolves versus leopards (r = 0.18, df = 70, $P=0.85$).

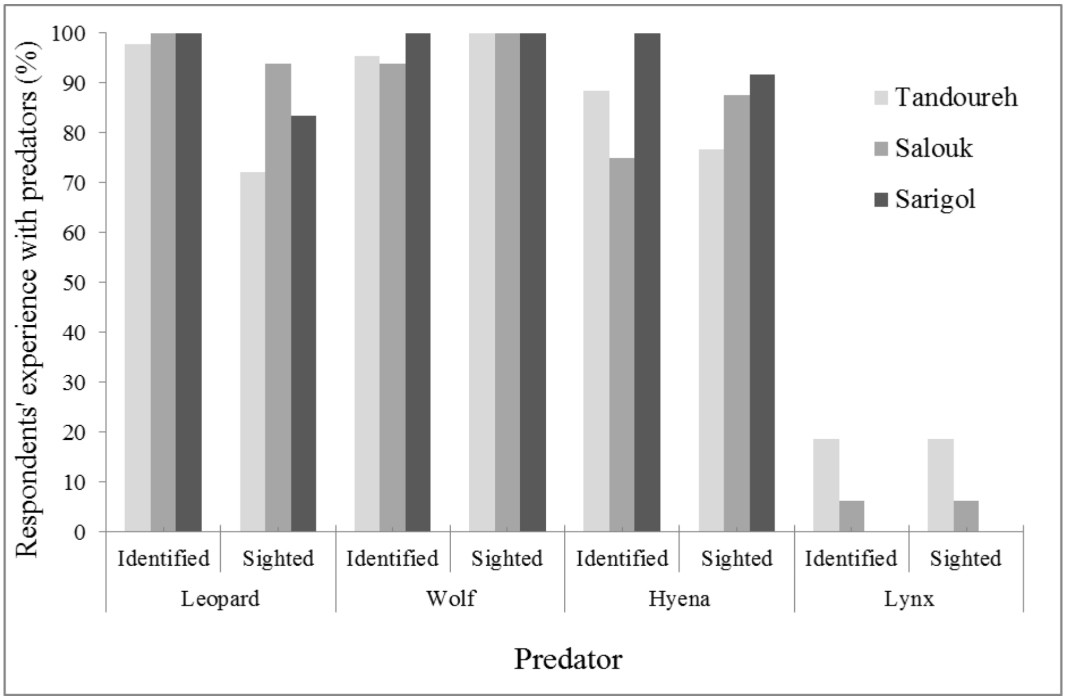

Fig 3.2 Percentage of interviewees who has been able to identify the species based on indicated image (labeled as "identified") and those who saw the animal in the wild (denoted as "sighted").

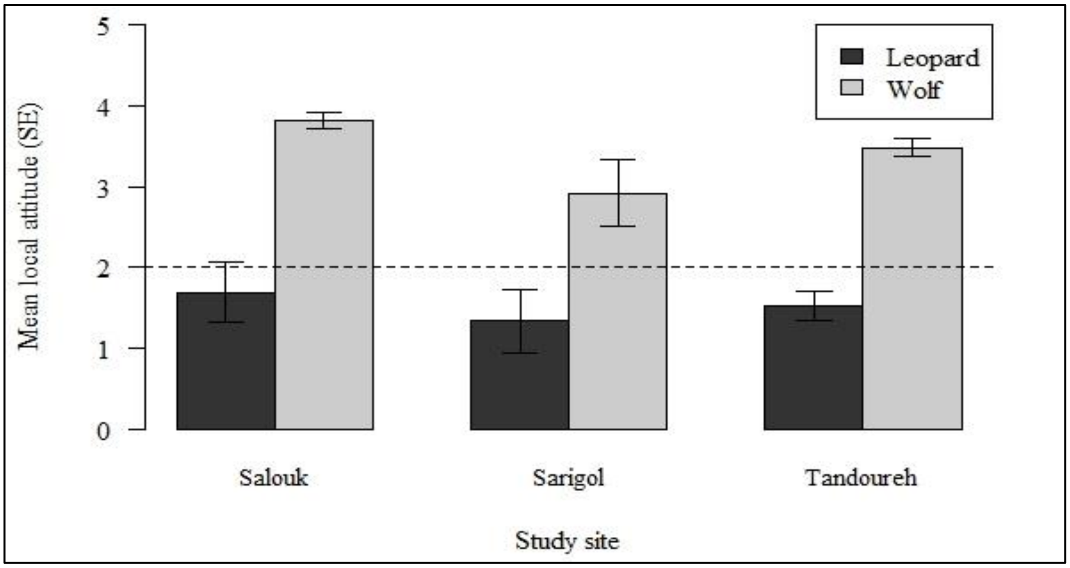

Fig 3.3 Comparative mean attitude of herders toward large carnivores in charge of killing livestock around multiple study sites in northeastern Iran. The score ranges from 0 (most positive) to 4 (most negative), with 2.0 neutral (dotted line). Error bars represent standard error for each mean.

There was evidence that perceived leopard predation on livestock differed between reserves and seasons. It was reported to be least prevalent in Tandoureh (GLMM, Z=-2.186, *P*= 0.028). More than half of leopard attacks occurred during spring in all surveyed reserves. In contrast, there was no evidence that the rate of reported wolf attacks varied among reserves (Table 3.2). There was evidence that losses to wolves varied with season, peaking in summer (GLMM, Z=3.358, *P*<0.001) and lowest in winter (Z=-4.263, *P*<0.001). There was no evidence that the seasonal patterns for either predator differed among reserves (the interaction between seasons and reserves on livestock loss was non-significant for both predators).

Table 3.2 Results of GLMM of effect of season and reserve on predatory events committed by the a) leopards and b) wolves in northeastern Iran. The reference level for season and reserve is respectively autumn and Salouk, so these estimate values are the difference from the reference levels. No significant interaction between seasons and reserves on livestock loss due to each predator was seen. * =$P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

a) Leopard

Random effects: Groups	Name	Variance	SD		
Respondent: Village	(Intercept)	1.577	1.2559		
Village	(Intercept)	0.214	0.4626		
Fixed effects:	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-2.5072	0.6432	-3.898	9.69E-05	***
ReserveSarigol	-0.1294	0.7286	-0.178	0.859069	
ReserveTandoureh	-1.4024	0.6417	-2.186	0.028845	*
SeasonSpring	1.4553	0.4196	3.468	0.000524	***

SeasonSummer	0.452	0.4833	0.935	0.349713
SeasonWinter	0.2513	0.5038	0.499	0.617882

b) Wolf

Random effects: Groups	Name	Variance	SD
Respondent: Village	(Intercept)	0.4118	0.6417
Village	(Intercept)	0.1888	0.4345

Fixed effects:	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	0.2984	0.2945	1.013	0.310888	
ReserveSarigol	-0.5428	0.4077	-1.331	0.18304	
ReserveTandoureh	-0.3397	0.3496	-0.972	0.331207	
SeasonSpring	-0.1488	0.1478	-1.007	0.313862	
SeasonSummer	0.4334	0.1291	3.358	0.000786	***
SeasonWinter	-0.7591	0.1781	-4.263	2.02E-05	***

During the one year period targeted by the questionnaire survey, a total of 2,172 domestic sheep and goats were reported to have died to non-predation causes. On average, each herder reported losing 26.8 ± SE 6.6 (Sarigol), 27.7 ± SE 4.2 (Tandoureh) to 39.6 ± SE 10.7 (Salouk) domestic sheep and goat as a result of non-predatory factors per annum (Fig 3.4). The respondents were not aware of type of disease for 33.7% of losses, but they allegedly assigned five different diseases to have resulted in death of livestock, based on local vet diagnosis (Fig 3.4 and Table 3.3). Thieves, loss, and catastrophes comprised a negligible part of the local non-predatory economic loss (<2%).

Table 3.3 Details of different causes of non-predatory mortality, particularly diseases among local stock between August 2013 to September 2014 in northeastern Iran.

Mortality cause	**Tandoureh**	**Sarigol**	**Salouk**	**Total**	**Percentage**
Foot-and-mouth disease (FMD)	125	49	158	332	15.3
Theileriosis	20	0	51	71	3.3
Enterotoxaemia	8	5	0	13	0.6
Peste des petits ruminants (PPR)	49	22	0	71	3.3
Still-birth*	440	170	282	892	41.1
Black disease	0	46	0	46	2.1
Unknown	569	36	127	732	33.7
Lost	14	0	1	15	0.7
Total	1,225	328	619	2,172	

* Still-birth, a common problem among local stock, can be mediated by various causes such as Bovine Viral Diarrhea, Toxoplasmosis, Enzootic Abortion of Ewes (EAE) and Campylobacte. But our interviewees were not aware of causes, calling all cases of mortalities between birth up-to first month a generally as “still-birth”.

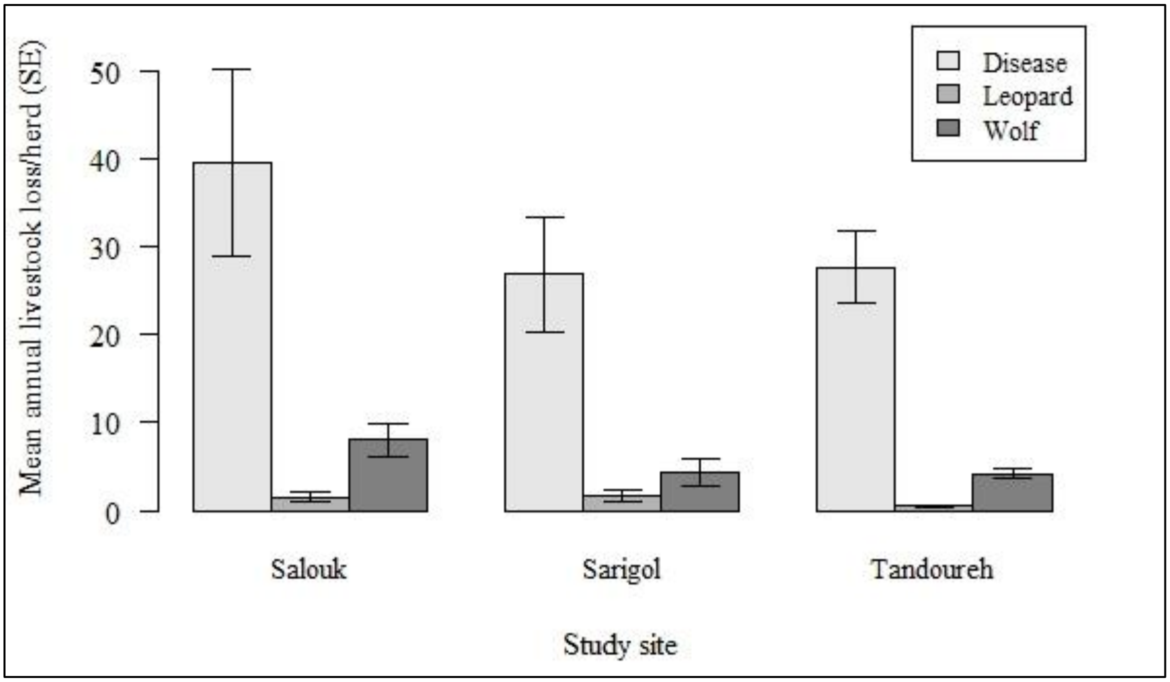

Fig 3.4 Mean annual livestock loss per each herd mediated by three main sources of mortality, i.e. diseases or depredation by wolves or leopards. Error bars represent standard error for each mean.

Reported losses due to non-predatory causes differed between seasons, peaking in winter (GLMM, Z=22.613, *P*<0.001) and spring (GLMM, Z=17.524, *P*<0.001). However, studied reserves showed no significant spatial difference in total annual loss. Non-predatory causes of mortality accounted for many more losses than all predation combined (predatory causes: 6.0 ± SE 0.7 vs. non-predatory causes 30.2 ± SE 3.6, t=-6.54, df = 75.96, *P*<0.001, Fig 3.4).

3.4.4. Factors affecting perceived loss and attitudes

There was no evidence that herding practices (either herd sizes or the number of dogs used) affected reported livestock loss numbers to either leopards or wolves (Table 3.4). Livestock loss was identified as a possible influence on attitudes. The seven top models ($\Delta AIC_c < 2$) for predicting attitude to leopards contained livestock loss due to wolves (WolfLoss) as a predictor. The five models with this predictor had clearly the highest weight of those compared (cumulative AICc weight: 0.61). Higher perceived loss to wolves was associated with more negative views toward leopards with the largest relative importance within top models (Fig 3.5 & Table 3.5). The maximum likelihood parameter estimate for this predictor in the best performing model was positive and with a confidence interval excluding zero (Fig 3.5 & Table 3.6). None of the other factors examined had a larger influence on attitudes towards leopards. There was little evidence that any of the predictors explored were associated with respondents' attitude toward wolves, including perceived losses to wolves (Table 3.5).

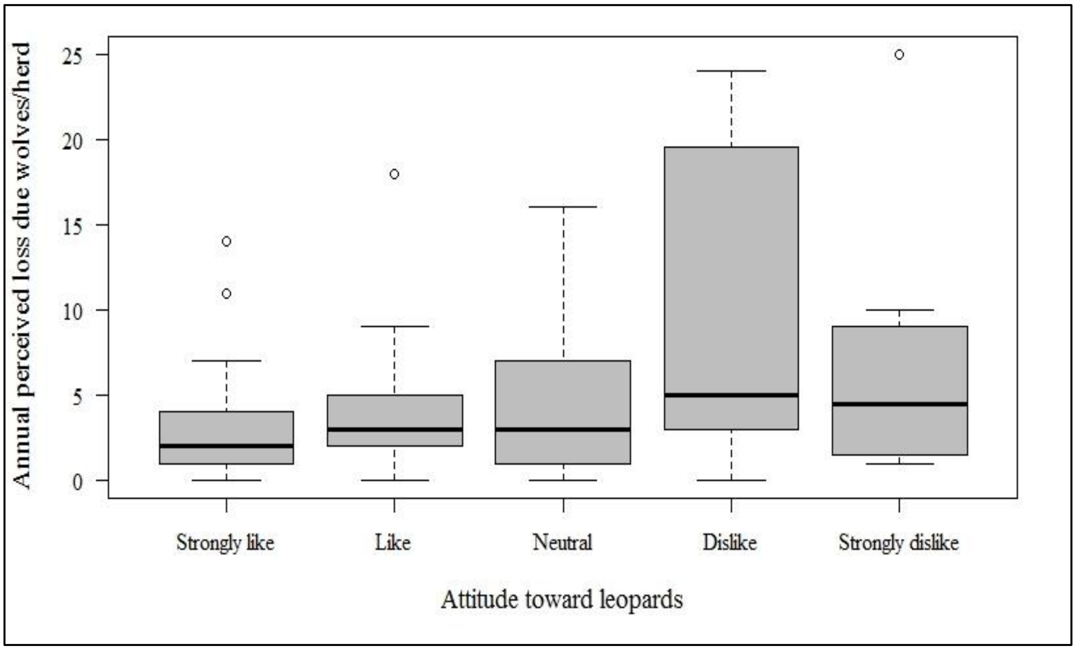

Fig 3.5 Median effect of livestock loss caused by the wolves on the respondents' attitudes toward the leopards in northeastern Iran. Each box represents inter-quartile range whereas whiskers delineate scores outside the inter-quartile range.

Table 3.4 Results of GLMM of herding practices on predation events attributed to a) leopards and b) wolves in northeastern Iran.

a) Leopard

Random effects: Groups	Name	Variance	SD
Village	(Intercept)	0.7717	0.8785

Fixed effects:	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	0.17138	0.46712	0.367	0.7137	
ReserveSarigol	-0.20205	0.63082	-0.320	0.7487	
ReserveTandoureh	-1.35735	0.61882	-2.193	0.0283	*
Z_Herdsize	-0.45266	0.25181	-1.798	0.0722	
Z_Dog	-0.30324	0.21402	-1.417	0.1565	
Z_DiseaseLoss	0.05663	0.18424	0.307	0.7585	

b) Wolf

Random effects: Groups	Name	Variance	SD
Village	(Intercept)	0.3441	0.5866

Fixed effects:	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	1.72583	0.27881	6.190	6.02e-10	***
ReserveSarigol	-0.44578	0.38154	-1.168	0.243	
ReserveTandoureh	-0.23151	0.34065	-0.680	0.497	
Z_Herdsize	-0.02966	0.08717	-0.340	0.734	
Z_Dog	0.09318	0.08234	1.132	0.258	
Z_DiseaseLoss	0.07479	0.04860	1.539	0.124	

Table 3.5 Results of GLMM of factors affecting attitudes towards leopard and wolf in northeastern Iran.

Model number	Model	K	AICc	AICc delta	AICc Weight	Cum. AICc Weight
	Leopard					
1	Attitude ~ Reserve + WolfLoss	2	224.70	0.00	0.19	0.19
2	Attitude ~ Reserve + WolfLoss + Price	3	224.80	0.10	0.18	0.37
3	Attitude ~ Reserve + Age + LeopardLoss	3	225.95	1.25	0.1	0.48
4	Attitude ~ Reserve + Age + WolfLoss	3	226.26	1.56	0.09	0.56
5	Attitude ~ Reserve + LeopardLoss + Price	3	226.30	1.61	0.09	0.65
6	Attitude ~ Reserve + Herdsize + WolfLoss	3	226.64	1.94	0.07	0.72
7	Attitude ~ Reserve + LeopardLoss + WolfLoss	3	226.64	1.94	0.07	0.8
8	Attitude ~ Reserve + LeopardLoss	2	227.10	2.40	0.06	0.85
9	Attitude ~ Reserve + Price	2	228.23	3.53	0.03	0.89
10	Attitude ~ Reserve	1	228.45	3.75	0.03	0.92
11	Attitude ~ Reserve + Herdsize +LeopardLoss	3	229.06	4.36	0.02	0.94
12	Attitude ~ Reserve + Age + Price	3	229.44	4.74	0.02	0.95
13	Attitude ~ Reserve + Age	2	229.64	4.94	0.02	0.97
14	Attitude ~ Reserve + Herdsize + Price	3	230.23	5.53	0.01	0.98
15	Attitude ~ Reserve + Herdsize	2	230.44	5.74	0.01	0.99
16	Attitude ~ Reserve + Age + Herdsize	3	231.64	6.94	0.01	1
	Wolf					
1	Attitude ~ Reserve	1	149.8	0	0.19	0.19
2	Attitude ~ Reserve + Price	2	150.9	1.06	0.11	0.3
3	Attitude ~ Reserve + Price + DiseaseLoss	3	151.4	1.57	0.09	0.39
4	Attitude ~ Reserve + DiseaseLoss	2	151.5	1.69	0.08	0.47
5	Attitude ~ Reserve + WolfLoss	2	151.8	1.98	0.07	0.54

6	Attitude ~ Reserve + Herdsize	2	152.2	2.39	0.06	0.6
7	Attitude ~ Reserve + Age	2	152.3	2.48	0.05	0.65
8	Attitude ~ Reserve + Price + WolfLoss	3	153.1	3.25	0.04	0.69
9	Attitude ~ Reserve + Age + Price	3	153.4	3.58	0.03	0.72
10	Attitude ~ Reserve + Price + Herdsize	3	153.4	3.6	0.03	0.75
11	Attitude ~ Reserve + DiseaseLoss + WolfLoss	3	153.5	3.65	0.03	0.78
12	Attitude ~ Reserve + Price + Disease Loss + WolfLoss	4	153.6	3.73	0.03	0.81
13	Attitude ~ Reserve + Age + DiseaseLoss + Price	4	154	4.16	0.02	0.83
14	Attitude ~ Reserve + Herdsize + DiseaseLoss	3	154.1	4.24	0.02	0.85
15	Attitude ~ Reserve + Age + DiseaseLoss	3	154.1	4.26	0.02	0.87
16	Attitude ~ Reserve + Price + Herdsize + DiseaseLoss	4	154.1	4.28	0.02	0.89
17	Attitude ~ Reserve + Herdsize + WolfLoss	3	154.3	4.48	0.02	0.91
18	Attitude ~ Reserve + Age + WolfLoss	3	154.4	4.57	0.02	0.93
19	Attitude ~ Reserve + Age + Herdsize	3	154.8	4.98	0.02	0.95
20	Attitude ~ Reserve + Herdsize + Price + WolfLoss	4	155.7	5.88	0.01	0.96
21	Attitude ~ Reserve + Age + Price + WolfLoss	4	155.7	5.88	0.01	0.97
22	Attitude ~ Reserve + Age + Herdsize + Price	4	156.1	6.24	0.01	0.98
23	Attitude ~ Reserve + Herdsize + WolfLoss + DiseaseLoss	4	156.1	6.32	0.01	0.99
24	Attitude ~ Reserve + Age + WolfLoss + DiseaseLoss	4	156.2	6.33	0.01	1
25	Attitude ~ Reserve + Age + Herdsize + DiseaseLoss	4	156.7	6.91	0.01	1
26	Attitude ~ Reserve + Age + Herdsize + WolfLoss	4	157	7.18	0.01	1

Table 3.6 Maximum likelihood estimates corresponding to the best performing AICc model: Attitude toward Leopards ~ Reserve + WolfLoss.

Parameter	**Estimates from best performing model** *Attitude toward Leopards ~ Reserve + WolfLoss*	
	Coefficient estimate	**Standard error**
WolfLoss	0.54	0.22

3.5. Discussion

The livestock herders we surveyed appear to have had a sophisticated understanding of the causes of livestock mortality. They believed their economic loss due to predation by large carnivores was low in absolute terms, and much lower than losses to non-predatory causes. Further, herders made fine-scale distinctions between the impacts of different carnivores, regarding the wolf as the main nuisance animal to livestock, regardless of peoples' recent experiences. In contrast, the leopard was reported to be responsible for a small proportion of herders' recent losses (0.4%) from August 2013 to September 2014, well below typical values reported for wild felids regionally (Babrgir et al., 2017; Dar et al., 2009; Kabir et al., 2013) or globally (up to 3% of annual domestic stocks; Nowell and Jackson, 1996). Despite this high level of understanding and a general pattern of more positive attitudes to leopards, we showed that perceived losses to wolves have a negative effect on attitudes to leopards.

3.5.1. Attitudes and perception

Attitudes toward wolves were generally negative, regardless of occurrence and intensity of livestock loss mediated by the predator. Stockholders were questioned only about successful wolf attacks on livestock. However, many herders noted that wolves were frequent visitors but were apparently deterred by guard dogs and shepherds. We were unable to quantify unsuccessful predation events - stockholders rarely remembered the details of these. Besides economic costs, predation on livestock poses a hidden impact incurred through the cost of guarding practices (Barua et al., 2013) which can lead to more negative attitudes.

The lower economic impact of leopards compared with wolves probably promotes more positive attitudes. The fact that leopards are responsible for many more attacks on humans does not seem to outweigh this which appears unexpected. Conflict with wolves was the main determinant of people's attitude toward the leopards. People experiencing more loss to wolves tended to have more negative attitudes toward leopards.

The primary reason that people are relatively tolerant of leopards compared with wolves is economic; both perceived losses and the costs of guarding are influential. Leopard attacks clearly followed a seasonal pattern, coinciding with the main grazing season in highlands. However, guarding is necessary throughout the year, as a response to year-round predation risk in lowlands by wolves. This may contaminate attitudes towards predators which have negligible economic impact.

Neither herd size nor number of accompanying dogs were associated with livestock loss. In contrast, higher levels of livestock depredation in warm seasons when local herds graze in remote mountainous areas support the idea that increased availability at this time triggers predation by the leopards. As has been observed in previous surveys in prey-rich landscapes (Farhadinia et al., 2014; Ghoddousi et al., 2016), limited spatiotemporal accessibility of domestic ungulates with basic herding practices can result in infrequent depredation by the leopards.

3.5.2. Conclusions

In a mosaic landscape hosting a guild of large carnivores, an understanding of how attitudes to different species interact in human communities may help conservation planning, especially for conservation-dependent species such as the Persian leopard. Our research calls for measures to reduce conflict not only with the threatened species, i.e. Persian leopard, but also with the more resilient carnivore, i.e. wolf.

Disease is perceived as the main economic threat to livestock-based livelihoods in this part of the world (Dar et al., 2009; Hosseini-Zavarei et al., 2013) and focusing on interventions to reduce its real and perceived burden would likely benefit both people and carnivores. Most importantly, the provision of veterinary services to control disease in livestock, particularly for stillbirth cases which comprise the largest proportion of livestock mortality, may increase people's tolerance toward the large carnivores, particularly if these veterinary initiatives are explicitly deployed as part of a conservation program (Nawaz et al., 2016). Compensation payment methods, in terms of conservation impacts, have an ambiguous effect in reducing the livestock depredation by large carnvires (Dickman et al., 2011). An effective insurance program must compensate for losses to wolves, not only leopards, to encourage more peaceful human–leopard coexistence. Finally, maintenance of the current exclusive grazing pattern for wild and domestic ungulates is necessary, boosted by anti-poaching attempts to secure the leopard populations within the current network of national parks.

3.6. References

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Chapter 4:

Anchoring and adjusting amidst humans: ranging behavior of Persian leopards along Iran-Turkmenistan borderland

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Anchoring and adjusting amidst humans: ranging behavior of Persian leopards along the Iran-Turkmenistan borderland

4.1. Abstract

Understanding the space use and movement ecology of apex predators, particularly in mosaic landscapes encompassing different land-uses, is fundamental for formulating effective conservation policy. The top extant big cat in the Middle East and the Caucasus, the Persian leopard *Panthera pardus saxicolor,* has disappeared from most of its historic range. Its spatial ecology in the areas where it remains is almost unknown. Between September 2014 and May 2017, we collared and monitored six adult leopards (5 males and 1 female) using GPS-satellite Iridium transmitters in Tandoureh National Park (355 km^2) along the Iran-Turkmenistan borderland. Using auto-correlated Kernel density estimation based on a continuous-time stochastic process for relocation data, we estimated a mean home range of 103.4 ± SE 51.8 km^2 for resident males which is larger than has been observed in other studies of Asian leopards. Most predation events occurred in core areas, averaging 32.4 ± SE 12.7 km^2. Although neighboring leopards showed high spatiotemporal overlap, their hunting areas were largely exclusive. Five out of six of leopards spent some time outside the national park, among human communities. Our study suggests that a national park can play an ‘anchoring’ role for individuals of an apex predator that spend some time in the surrounding human-dominated landscapes. Therefore, we envisage that instead of emphasizing either land sharing or land sparing, a combined approach can secure the viability of resilient large carnivores that are able to coexist with humans in the rugged montane landscapes of west and central Asia.

Keywords: ranging behavior, satellite GPS telemetry, Persian leopard, land sparing, land sharing, auto-correlated kernel distribution estimator.

4.2. Introduction

Wide-ranging apex predators have spatial needs that may push them to wander beyond the boundaries of protected areas (Balme et al., 2010; Johansson et al., 2016). Prey availability and environmental productivity are major factors driving predator space use (Loveridge et al., 2009; Nilsen et al., 2005). Predator movement patterns are also regulated by their population density (Balme et al., 2010; Benson et al., 2006; Goodrich et al., 2010) and climatic disturbance in resource availability (Allen et al., 2016). Ranging beyond protected areas' boundaries is often associated with higher human-induced mortality (Balme et al., 2010; Swanepoel et al., 2015).

The high altitude areas of west and central Asia host low densities of wild ungulates, predominantly confined to protected areas, while high number of domestic animals dominate montane pastures (Baskin and Danell, 2003; Mallon and Zhigang, 2009). These crowded landscapes with high spatiotemporal variability in resources create formidable challenges for conservation managers attempting to reduce conflict and foster coexistence between humans and top predators.

Current conservation planning for landscapes dominated by humans has elements of two main paradigms, the “coexistence” (land sharing) versus the “separation” (land sparing) models. The “land sharing” model, in which carnivores and humans inhabit shared landscapes, is believed to have facilitated the recovery of large carnivores in Europe (Chapron et al., 2014) and North America (Gompper et al., 2015). In contrast, “land sparing” through separating areas for human use from those for wildlife conservation is shown to be more efficient for a wide range of taxa and landscapes, from trees and birds (Phalan et al., 2011) to the African lion *Panthera leo* (Packer et al., 2013). Sparing such extensive tracts for large carnivores is unlikely to be possible in many parts of the world, particularly in dry areas where these animals need to range widely to access unpredictable and spatially heterogeneous resources (Durant et al., 2015).

Large cats living in the mountains of Asia, where they are threatened by habitat loss and persecution (Jacobson et al., 2016; McCarthy et al., 2017), persist at low density (usually <3 individuals/100 km^2 (Alexander et al., 2015; Hamidi et al., 2014)) compared to other productive landscapes (Karanth et al., 2004; Tobler and Powell, 2013). In human-dominated montane landscapes of Asia, land sharing is essential for persistence of large felids (Johansson et al., 2016). Nonetheless, to the best of our knowledge, there is no study which has evaluated the role of established protected areas in west and central Asia’s rugged terrain, and how the current debate of land sparing and/or land sharing is relevant for the viability of large cats in a mosaic of spared/shared lands. We address this challenge with a GPS telemetry study of the Persian leopard *P. pardus saxicolor* in Tandoureh National Park along the Iran-Turkmenistan borderland.

Currently, only a small percentage of the extant range of leopards lies within the current network of protected areas (Jacobson et al., 2016). Importantly, many Asian borderlands harbour fragmented populations of leopards (Farhadinia et al., 2015; Jiang et al., 2015; Rostro-García et al., 2016) without effective transboundary conservation programs (Jacobson et al., 2016). Addressing these two challenges, i.e. low protected area coverage and the lack of international cooperation is partly dependent upon a thorough understanding of the spatial and movement ecology of leopards at various political scales.

Leopards vary widely in spacing patterns across their global range influenced by densityand the predictability of resources (Nilsen et al., 2005; Stein and Hayssen, 2013). Inter-individual variations, according to age, gender (Bailey, 1993; Balme et al., 2010; Mizutani and Jewell, 1998) and reproductive status (Rozhnov et al., 2015) cause differences in ranging behaviour. Humans also can indirectly influence the spatial ecology of leopards by creating a vacuum effect whereby territories made vacant by human action cause movement from adjacent territories (Balme et al., 2009). Decreased population density mediated by human-induced mortality can also result in inter-sexual differences in the spacing dynamics of leopards (Fattebert et al., 2016). There is also a negative relationship between habitat productivity (as a proxy for prey abundance) and home range size in leopards, i.e. home-range size decreases as productivity increases (Nilsen et al., 2005). Precipitation indirectly affects the ranging behavior via its influence on primary productivity and prey biomass (Marker and Dickman, 2005).

In this paper, we used GPS data to address three objectives concerning the ranging behavior and movement ecology of Persian leopards, the top predator along the Iran-Turkmenistan borderland. We provided the first robust home range estimates for leopards in the steppe mountains of Asia. We then quantified the degree of range overlap between conspecifics of the same sex. Finally, we explored how the boundaries of a national park affected leopards’ use of space. Besides improving our understanding of leopard movement ecology and ranging behavior within a mosaic landscape of human-dominated areas and human-free national park, our findings are relevant for better management of many montane areas, where islands of small reserves are surrounded by densely populated human areas.

4.3. Materials and Methods

4.3.1. Ethics statement

The study was conducted in Tandroueh National Park, Iran. The Iranian Department of Environment reviewed all sampling, trapping and handling procedures and approved permits for the work conducted (93/16270). The trapping and handling protocol was also approved by the University of Oxford's Ethical Review Committee (BMS-ERC-160614).

4.3.2. Study Area

We studied leopards in Tandoureh NP, north-eastern Iran (ca. 20 km from the Turkmenistan border) from September 2014. The park has been protected since 1968 and covers 355 km^2. It is characterized by mountains covered with wormwood *Artemisia* sp. and scattered juniper trees *Juniperus* sp. Elevation and annual precipitation range from 1,000 to 2,600 m and 250 to 300 mm, respectively.

There is no human settlement inside the park. Besides the two main cities, i.e. Dargaz (Iran) and Ashgabat (the capital of Turkmenistan) near our study area (Fig 1), local communities live in villages with population ranging between 30 to 400 households. They are mainly sheep and goat herders.

The main prey species for leopards include urial *Ovis orientalis*, bezoar goat *Capra aegagrus*, and wild pig *Sus scrofa*. The availability of leopard prey in Tandoureh is affected by the national park boundaries. Wild medium-sized prey are available only inside the park, whereas domestic animals are found exclusively outside the park. The only exceptions are wild pigs, which are occasionally found in multi-use areas, outside the national park.

4.3.3. Leopard capturing and collaring

We captured leopards with Aldrich foot-snares extensively modified to reduce chances of injury (Frank et al., 2003) and remotely monitored with VHF trap transmitters (Wildlife Materials, Inc., Illinois, USA) every 1-2 hours. As leopards are known to respond to baits, a wild pig carcass was used as bait, normally hanging from a tree or rock. Traps were also deployed along trails leading to the baits. In summer, we deployed traps along trails leading to water sources, sometimes without bait (Farhadinia et al., 2017).

We immobilized leopards using a combination of ketamine 10% (Alfasan, Nederland BV) 2 mg/kg, medetomidine HCl 20 mg/ml (Kyron Laboratories (Pety) Ltd., Johannesburg, South Africa) 30 µg/kg and butorphanol 0.2 mg/kg (Torbugesic®, Fort Dodge Animal Health Fort Dodge Animal Health, Iowa 50501 USA) delivered intramuscularly with a dart gun (Daninject, Denmark) using a 1.5 ml dart. Trapping and handling were undertaken following approval by the University of Oxford’s Ethical Review Committee (Zool-AWERB-16062014) and the Iranian Department of Environment (93/16270).

We used GPS collars with Iridium download (LOTEK Engineering Ltd., Newmarket, ON, Canada). Each collar incorporated a drop-off buckle with a timer set to 52 weeks since deployment. Collars weighed 640 g, equivalent to less than 1-2% of leopard body mass.

Age estimates were based on dental features (Stander, 1997). Anesthesia lasted for 44 to 60 minutes, followed by reversal using atipamazole (3 times the medetomidine dosage) and nantroxan (the doses equal to butorphanole), injected intramuscularly.

For programming the collars’ fix rates, we followed Knopff et al. (2009) who recommended recording fixes every 3 hours to enable the identification of spatially aggregated GPS points, or clusters. However, to increase fix success rates (Cain III et al., 2005) fixes were taken hourly during the last week of each month. Also, a ‘virtual fence’ option enabled us to upload the area’s boundary, so that when leopards left the defined area fix rate could be increased to hourly. Bjørneraas et al. (2010) recommended that to analyze animal movement and behavior, fixes obtained immediately after collaring should be excluded because the animal is likely to behave abnormally. Therefore, we omitted the first 4 days for all collar data, associated with the earliest known kill made by the leopards after collaring (M1/Borzou).

We also investigated the potential kill sites of collared leopards. Kills were defined by clusters of GPS fixes, i.e. locations where leopards remained overnight (6 PM to 6 AM) within a radius of 200 meters. Candidate GPS clusters were investigated for possible kill remains. Prey species were categorized as “small” as < 15 kg, including red fox *Vulpes vulpes*, Indian crested porcupine *Hystrix indica* and birds or “medium” as ≥ 15 kg, such as urial, bezoar goat, wild pig, domestic sheep *Ovis aries* and domestic dog *Canis familiaris*. Young wild ungulates and domestic animals (< 1 year) were also included in medium-sized prey.

4.3.4. Statistical analysis

We screened the data for two types of errors which are typical in GPS locations: missing location fixes (i.e. unsuccessful attempts of a GPS fix) and location errors of successfully acquired fixes (i.e. the difference between the recorded location and the animal's true location) (Bjørneraas et al., 2010). After removing missing fixes, erroneous locations and outliers were screened based on identification of locations arising from unrealistic movement patterns with minimal loss of data, using a script developed by Bjørneraas et al. (2010) implemented in the R environment for statistical computing (R Development Core Team, 2013). We defined conservative movement values for leopards as Δ=30,000 m; μ=15,000 m; α =5000 m/h; $\theta = -0.97$ corresponding to turning angles between 166° and d 194°; Δ is a distance threshold over which an individual could not possibly travel between consecutive intervals, μ is a distance that leopard can move between two fixes and α is speed.

Multiple home range estimators are suggested to facilitate comparison with other studies that use just one method. We used three estimators for quantifying home ranges of the leopards: minimum convex polygon (MCP), kernel density estimator (KDE) and auto-correlated KDE (AKDE). Both MCP and KDE are popular for estimating animals' home ranges, but they suffer from fundamental flaws that could degrade data quality. MCP lacks an underlying probabilistic model whereas the kernel is a nonparametric, probabilistic method, which calculates home range area based on the complete utilization distribution (UD, i.e., the probability distribution defining the animal's use of space [39]). . However, KDE assumes that the data are independent and identically distributed whereas relocation data that are ordered in time are inherently auto-correlated (i.e. an individual's position, velocity, or acceleration measured at one point in time are statistically correlated with the same measurements in the past and future). Therefore, we also used the recently developed AKDE method, a continuous-time approach which is a fully generalized KDE to account for auto-correlated bivariate Gaussian density estimation for relocation data (Fleming et al., 2015).

For each animal, we plotted an empirical variogram, which is the estimated semi-variance in positions as a function of the time lag separating observations to visually inspect the autocorrelation structure of the relocation data. Upward curvature at zero to short time lags indicates velocity autocorrelation while the long-lag behavior of the variogram illustrates space use. Thus, range residents are expected to reach an asymptote on a timescale that roughly corresponds to the home-range crossing time data (Calabrese et al., 2016). In the absence of proof of range residency, we excluded them from estimating population-level movement metrics.

We used package ‘ctmm’ version 0.4.0 (Calabrese et al., 2016) to perform three movement models. The Independent Identically Distributed (IID) process assumes uncorrelated positions and velocities which is equal to the conventional KDE (Fleming et al., 2015). The Ornstein–Uhlenbeck (OU) process combines a random search model without space use constraint (Brownian motion) with a tendency to remain in a particular home range. Finally, the Ornstein–Uhlenbeck Foraging (OUF) process features both velocity autocorrelation time scale (a measure of path sinuosity) and restricted space use (Calabrese et al., 2016; Fleming and Calabrese, 2017). Both the OU and OUF model processes accommodate auto-correlated data to estimate home range size and crossing time (day).

Starting values derived from semi-variograms were used for maximum likelihood model fitting. Suitable models were fitted to the data using maximum likelihood estimation and best models were selected based on their AICc weight. The best model for each individual leopard was used to calculate movement parameters and home range; the latter defined as area within 95% % UD isopleths of AKDE estimates.

Core areas of space use, defined as the area within which an animal spends a maximum amount of time, was estimated using an individual-based quantitative approach, following Vander Wal and Rodgers (2012). Thus, the AKDE utilization distribution area was plotted against isopleths to determine the point at which the proportional home range area begins to increase at a greater rate than the probability of use (slope=1). The value of the corresponding isopleth determines the boundary of the core area (Vander Wal and Rodgers, 2012). We then assessed the position of kills made by collared leopards in relation to the core area of their home ranges. We also calculated seasonal AKDE home ranges to explore variation in space use. Seasons were defined as spring= March to May, summer= June to August, fall= September to November and winter= December to February.

We used AKDE estimates to quantify an animal's utilization distribution (UD), i.e., the probability distribution defining the animal's use of space. Then we used AKDE's 'UD' option to illustrate the 'static interaction', i.e. the spatial overlap of 2 home-ranges and congruence in their utilization distributions (Mizutani and Jewell, 1998), ignoring the temporal sequence of movement paths (Fieberg and Kochanny, 2005). Our pairwise static interaction analysis was based only on those fixes obtained from the period of time when both animals were collared, partitioned by seasons. Therefore M4 was excluded from this analysis, because he was collared after other individuals' collars dropped off.

We calculated range overlap using function *overlap* in package 'ctmm' version 0.4.0 (Calabrese et al., 2016) which uses the Bhattacharya coefficient as an approximate measurement of the amount of overlap between two statistical samples. The overlap function incorporates movement models and calculates the overlap of their auto-correlated kernel density. For each pair of neighbors, we calculated the proportion of home range overlap of individual A on B and vice versa. A value of 1 implies that the two distributions are identical, while a value of 0 implies that the two distributions share no area in common.

In addition to home range and crossing time, two other movement parameters, the velocity autocorrelation time scale (a measure of path sinuosity) and mean distance travelled per day were also calculated (Calabrese et al., 2016; Fleming and Calabrese, 2017) by the OUF model. All statistical analysis were implemented in R environment for statistical computing (R Development Core Team, 2013).

4.4. Results

Between September 2014 and May 2017, we collared and monitored six leopards (5 males and 1 female) using GPS-satellite Iridium collars, comprising 4 adults and 2 young individuals in Tandoureh National Park. GPS collars collected between 54 and 368 days data per individual, representing a total of 56.7 monthly leopard study periods (Table 1). Our overall fix rate was high (mean 85.0% ± SE 7.6) and we obtained a total of 22226 GPS locations for 1702 leopard-days (283.7 ± SE 50.8 days/leopard). No erroneous fixes or spikes in movement were detected in our data, despite using very conservative movement parameters to screen location errors.

Overall, 17.9% of GPS fixes were located outside the park (Table 1). The five collared leopards which were observed outside the park varied substantially in the amount of time spent on multi-use lands (villages, farmlands and pastures), ranging between 2.2 to 43.8% (Fig. 1). Only the leopard M2/Bardia did not leave the park limits.

Between September 2014 and May 2017, we collared and monitored six leopards (5 males and 1 female) using GPS-satellite Iridium collars, comprising 4 adults and 2 young individuals in Tandoureh National Park. GPS collars collected between 54 and 368 days data per individual, representing a total of 56.7 monthly leopard study periods (Table 4.1). Our overall fix rate was high (mean 85.0% ± SE 7.6) and we obtained a total of 22226 GPS locations for 1702 leopard-

days (283.7 ± SE 50.8 days/leopard). No erroneous fixes or spikes in movement were detected in our data, despite using very conservative movement parameters to screen location errors.

Overall, 17.9% of GPS fixes were located outside the park (Table 4.1), five of the six collared leopards showed varied levels of home range overlap with human land use including villages, farmland and pastures (ranging between 2.2 to 43.8%; Fig 4.1). Only the leopard M2/Bardia did not leave the park limits.

Table 4.1 Movement parameters and home range estimates for GPS-collared leopards in Tandoureh National Park, northeastern Iran (2014-2017). Home ranges were estimated via 95% Kernel Density Estimates (KDE) and Autocorrelated Kernel Density Estimates (AKDE). Home ranges for individuals marked with asterisk (*) were based on an Ornstein-Uhlenbeck (OU) process model, while an Ornstein-Uhlenbeck Foraging (OUF) process model was fitted to the other animals. Models were selected based on their AICc weight as calculated by ctmm package. All means for ranging metrics are calculated after removing non-resident individuals (M1, F5 and M6).

Leopard Name/ID	**Sex/age**	**Capture date**	**Last day of fixes**	**Number of days**	**% days outside NP**	**Farthest fixes (km)**	**Home range crossing time (day)**	**Velocity autocorrelation timescale (h)**	**MCP 100% (km^2)**	**95% KDE (km^2)**	**AKDE (km^2) (95% CI)**	**Core area isopleths (%)**	**Core area (km^2) (95% CI)**	**% kills outside core area**
M1/Borzou*	M/+10	5.2.2015	4.2.2016	368	43.8	30.2	4.4	NA	475.7	417.6	563.4 (448.8-690.8)	60.0	224.5 (178.8-275.3)	13.6
M2/Bardia	M/8-10	3.10.2014	30.9.2015	362	0.00	9.8	0.3	0.2	63.3	43.6	43.9 (41.2-46.7)	61.8	16.5 (15.5-17.6)	18.2
M3/Borna*	M/5-6	28.9.2014	27.9.2015	364	13.5	28.8	1.2	NA	362.2	194.2	206.5 (186.6-227.3)	67.0	57.6 (51.2- 64.3)	8.7
M4/Tandoureh	M/7-10	16.8.2016	1.04.2017	228	2.20	15.3	0.6	0.1	113.9	56.8	59.8 (54.0-65.8)	61.7	23.1 (20.9-25.4)	5.3
F5/Iran	F/2-3	6.12.2015	29.1.2016	54	7.40	27.8	2.6	0.1	266.5	422.8	330.9 (208.9-480.6)	65.1	92.5 (58.4-134.3)	0.0
M6/Kaveh*	M/3-4	4.9.2015	26.8.2016	326	3.31	81.6	27.1	NA	1098.3	751.9	2269.0 (1262.4-3565.9)	62.3	775.7 (431.6-1219.0)	22.2
Mean (SE)				**283.7 (50.8)**	17.9 (7.3)				**179.8 (92.4)**	**98.2 (48.2)**	**103.4 (51.8)**	**63.5 (1.8)**	**32.4 (12.7)**	**10.7 (3.8)**

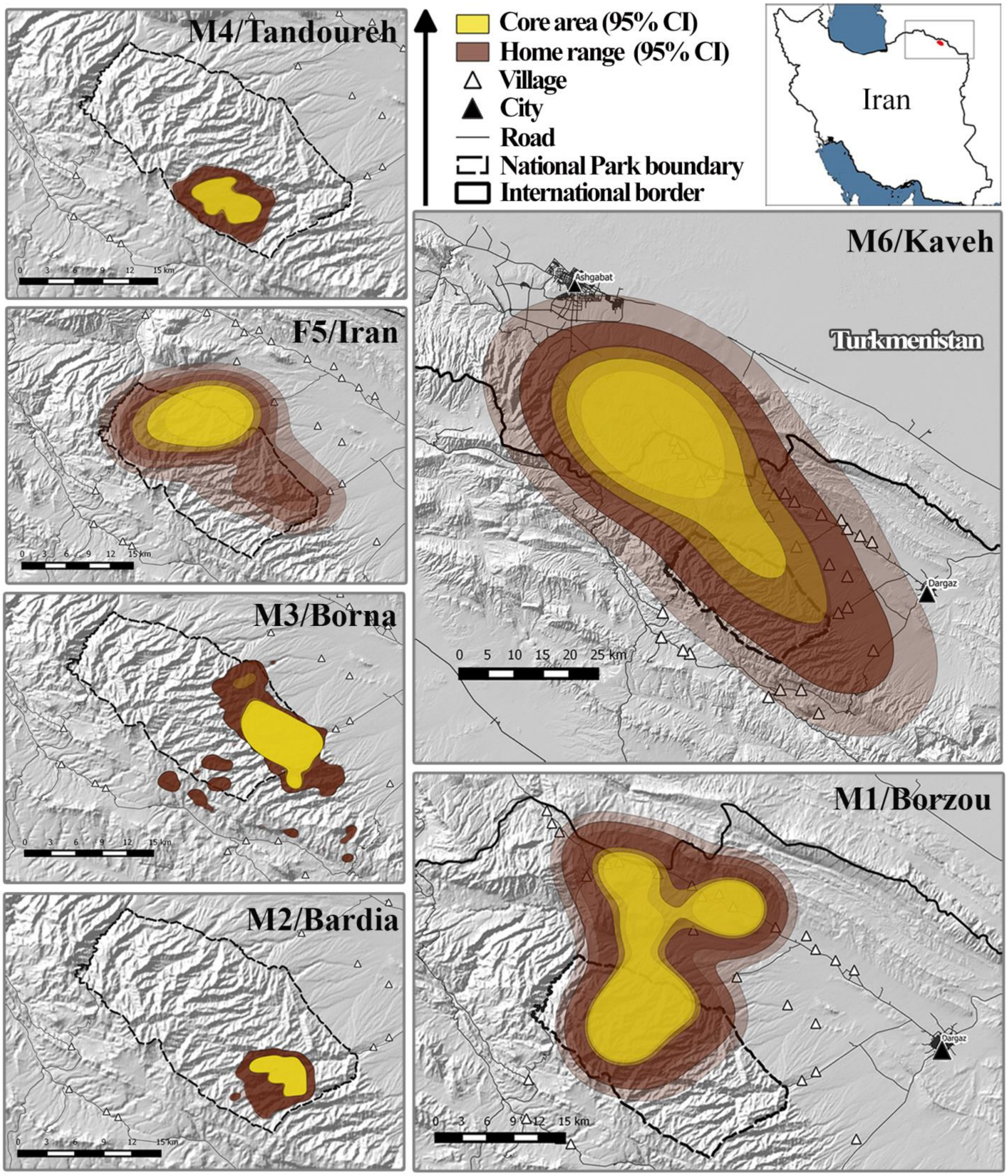

Fig 4.1 Home range of different leopard individuals tracked between September 2014 and May 2017 in Tandoureh National Park. Home ranges are delineated using auto-correlated Kernel density estimator (AKDE).

4.4.1. Home range size and overlap

Based on objective assessment of variograms (Fig 4.2), a clear asymptote was reached for three adult males M2, M3 and M4, showing their constrained space use as resident individuals. In contrast, both young leopards (F5 and M6) lacked an asymptote, evidence for lack of range residency. F5 was tracked for only 54 days which was probably not long enough to show range residency. M1 (old male) showed a mixed ranging pattern. He showed resident behavior until almost 5.5 months after collaring when his semi-variance increased and he started his excursions outside the park along the borderland's communities with regular returns to the national park.

We excluded non-resident individuals which did not constrain their space use (F5 and M6) and the old male (M1) that appeared to become a non-resident wandering animal from the estimates of home range size. Accordingly, mean AKDE home range was calculated to be 103.4 ± SE 51.8 km2 for resident males which was slightly larger than their non-correlated KDE home range size estimates (1.0 to 1.1 times; Table 4.1). M6, possibly a dispersing young male, showed the largest range use in one year, expanding from Iran into Turkmenistan, resulting in an elongated range with 81.6 km between farthest fixes (Table 4.1). His AKDE analysis revealed that he finally settled in Turkmenistan, according to his core area which was placed primarily within the Turkmen territory (Fig 4.1). This male had the largest difference between AKDE and KDE (AKDE > 3KDE). Mean estimated core area size for resident males was 32.4 ± 12.7 km2, which were represented by the 62% to 67% isopleths of the utility distribution (Table 4.1).

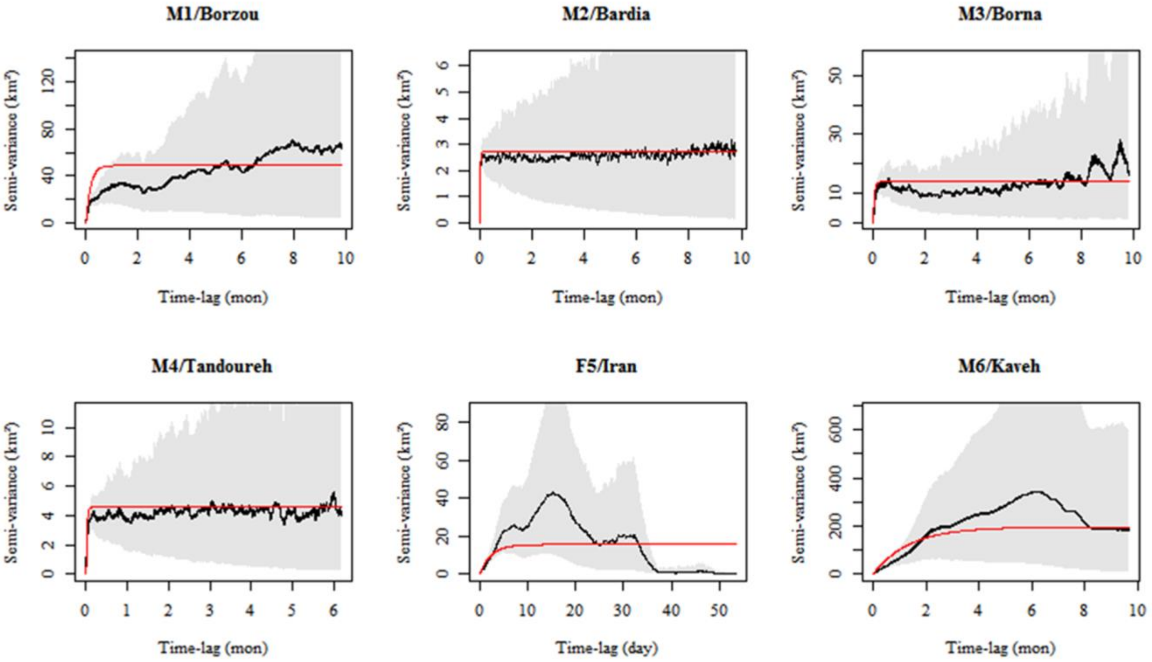

Fig 4.2 Objective assessment of residency in leopards, based on the variogram of each individual's observed movement track. For all individuals, the fraction of the variogram displayed is 80% of the duration of each dataset, except for F5/Iran which shows the entire collaring period, i.e. 54 days.

There was no consistent seasonal difference in AKDE home ranges for resident males ($F_{5, 6}$= 1.72, P = 0.26). Although our sample size was small, individual variations in seasonal home range size can be seen (Fig 4.3). The two resident males (M2 and M3) tended to have their smallest AKDE estimates during winter when snow covered higher elevations confine their ranging to lower areas (Fig 4.3 and 4.4).

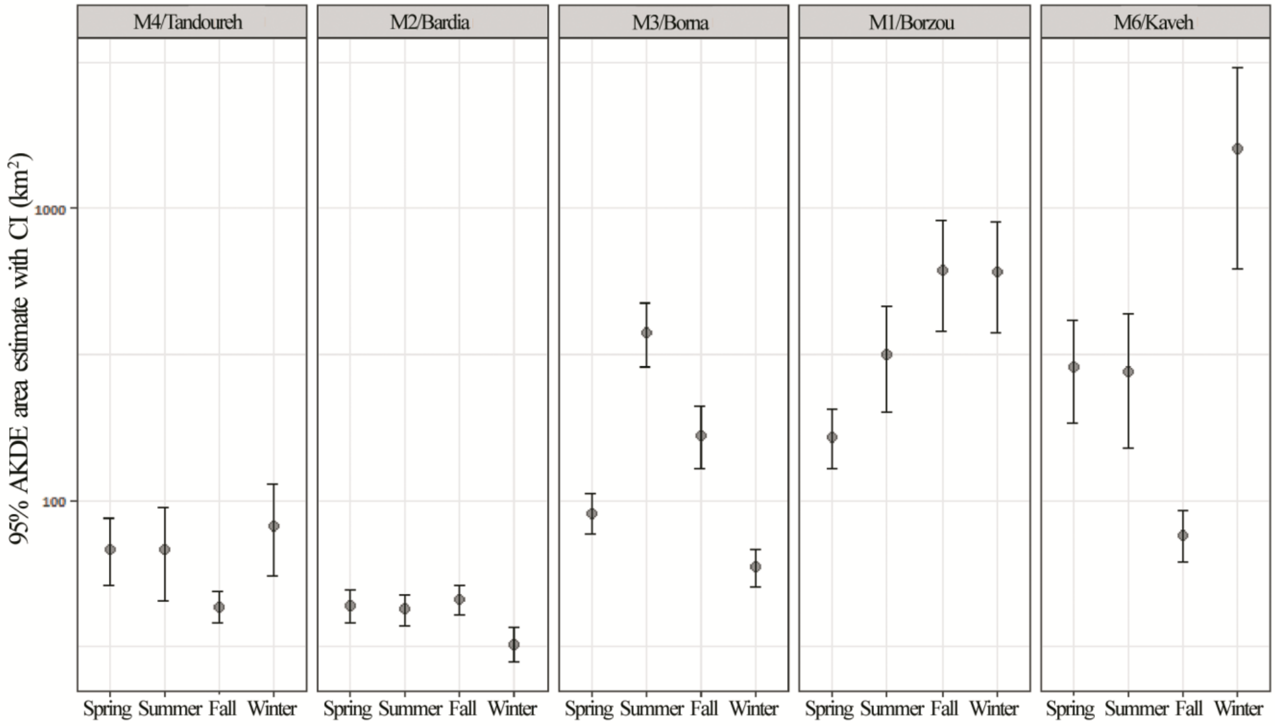

Fig 4.3 Seasonal home range of male Persian leopards analyzed as a continuous-time stochastic process using auto-correlated Kernel density estimator (AKDE). There is less variation in seasonal AKDE estimates for resident males (M2, M3 and M4) rather than transient individuals (M1 and M6).

Tau (home range crossing time), varied between 0.3 and 27.1 days, and was remarkably shorter for resident (M2, M3 and M4; 0.7 SE 0.3 days) than transient leopards (M1, F5 and M6; 11.3 SE 7.9 days; Table 4.1). The Ornstein-Uhlenbeck Foraging (OUF) process model scored higher for three leopards based on AICc values, resulted in low directionality in movement (velocity autocorrelation time scale; Table 4.1).

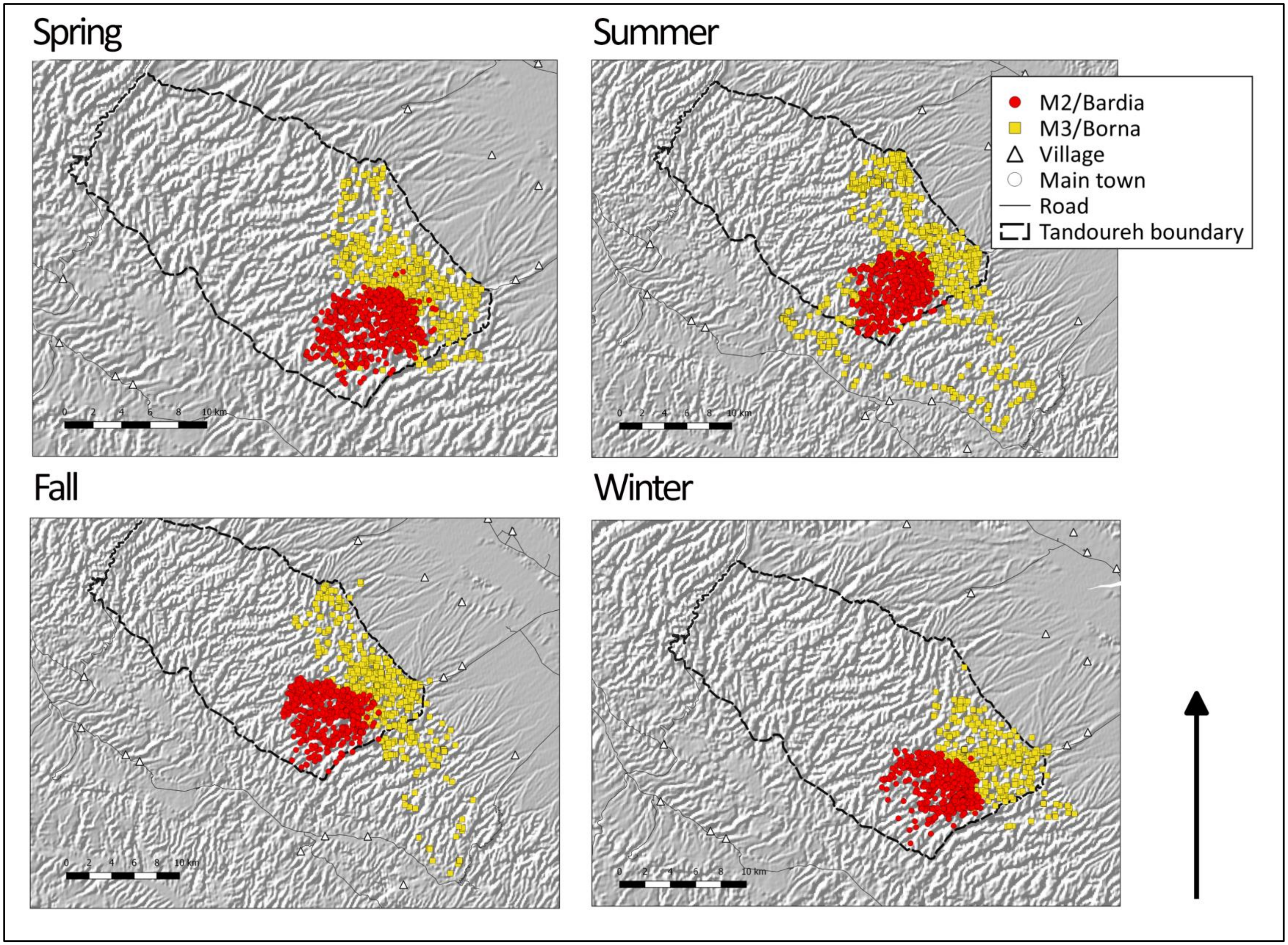

Fig 4.4 Seasonal fixes of two adult male Persian leopards (M2 and M3) in Tandoureh National Park.

In total, we found 139 kill remains where leopards preyed (n=130) or scavenged (n=9), belonging to 10 species, mostly medium sized prey (93.1%). On average, only 10.7 ± 3.8% of kills made by resident males were found outside the core areas of AKDE home ranges. No sign of predation or scavenging outside of the core area was found for the only collared female (F5/Iran) during her short tracking period while the largest proportion of outside core area's kills belonged to the M6, the young non-resident male (22.2%, Table 4.1).

All leopards with neighboring ranges showed moderate home range overlap, varying from 0.29 to 0.64 (mean= 0.43 SE 0.06; Table 4.2). Home range overlap was also similar between resident males (0.44 SE 0.10) and resident-transient individuals (0.39 SE 0.06).

Table 4.2 Mean pairwise home range overlap estimates (confidence intervals) between neighboring leopard individuals between September 2014 and May 2017 in Tandoureh National Park, northeastern Iran. M4 was excluded from the analysis because he was collared when all other collars were dropped off.

Home range overlap	**Borzou/M1 (resident/transient)**	**Bardia/M2 (resident)**	**Borna/M3 \(resident)**	**Iran/F5 (transient)**	**Kaveh/M6 (transient)**
Borzou/M1 (resident/transient)	NA	NA	0.64 (0.61-0.67)	0.49 (0.47-0.51)	0.29 (0.28-0.30)
Bardia/M2 (resident)	NA	NA	0.40 (0.38-0.42)	NA	NA
Borna/M3 (resident)	0.64 (0.61-0.67)	0.40 (0.38-0.42)	NA	NA	NA
Iran/F5 (transient)	0.49 (0.47-0.51)	NA	NA	NA	0.35 (0.33-0.36)
Kaveh/M6 (transient)	0.29 (0.28-0.30)	NA	NA	0.35 (0.33-0.36)	NA

4.5. Discussion

Our home range estimates for Persian leopards were larger than those reported in previous Asian leopard ranging studies. Range overlap between conspecifics was relatively high and the majority of predation events occurred within home range core areas. Excursions to areas occupied by people occurred on 17.9% of all leopard collaring days with very wide variation among individuals, pointing to the importance of a combining land sparing and land sharing approaches for leopard conservation.

4.5.1. Home range size

Although our data showed remarkable individual variation, leopards in Tandoureh occupied the largest home ranges recorded so far for Asian landscapes (Karanth and Sunquist, 2000; Odden and Wegge, 2005; Simcharoen et al., 2008), with the exception of an adult male tracked in an arid montane habitat in central Iran, i.e. 670 km^2 (Hunter, 2011). The home ranges of predators scale with body mass (Kelt and Van Vuren, 2001) and habitat productivity, which affects prey biomass (Nilsen et al., 2005). The large body masses of Persian leopards (Farhadinia et al., 2014) and the low primary productivity of the landscapes (e.g. annual rainfall 250-300 mm in northeastern Iran) are likely to be two key determinants of their larger home range sizes.

Seasonality may partly explain variation in leopard home-range sizes at the population scale (Nilsen et al., 2005). We found no evidence of consistent seasonal variation in home range size, in accordance with previous leopard studies (Fattebert et al., 2016; Marker and Dickman, 2005; Odden and Wegge, 2005; Simcharoen et al., 2008). Nonetheless, our data is consistent with previous observations (Marker and Dickman, 2005; Simcharoen et al., 2008) in suggesting that seasonal variation in home range size is an individual behavior rather than a population level trait. Both adult males (M2 and M3) restricted their ranging to lower elevations during winter when higher elevations are covered with snow and are extremely cold, both being factors known to be constraints for leopard habitat selection (Farhadinia et al., 2015; Gavashelishvili and Lukarevskiy, 2008).

4.5.2. Home range overlap

Leopards showed substantial home range overlaps in Tandoureh. The estimates of home range overlaps in the current study were considerably larger than reported in many previous studies on leopards (Table 4.3). There are two possible explanations. The majority of previous studies were based on VHF telemetry, which may miss significant animal movements and consequently result in smaller home range overlap estimates (Kochanny et al., 2009). Likewise, the conventional KDE and MCP estimation generally provide a lower bound for the estimate of home range area (Fleming et al., 2015), and consequently result in overlap reduction. Alternatively, the higher home range overlap observed in Tandoureh is attributable to the topographic features of this rugged landscape that can facilitate co-existence of multiple individuals. Landscape heterogeneity and topographic features can provide restricted detectability for leopards and promote their spatiotemporal overlap.

Predation occurred mostly in parts of the home range used exclusively by each leopard, i.e. home range core areas. We know of only two other studies evaluating the spatial configuration of hunting grounds in regard to felids' core areas. Predation events were reported to be more frequent outside core areas for both other case studies, i.e. jaguar *Panthera onca* (de Azevedo and Murray, 2007) and puma *Puma concolor* (Pierce et al., 2000). Amongst home ranges with high degrees of spatial overlap, exclusive hunting areas can facilitate coexistence of multiple individuals. Competition over resources, including kills, is a cause of intraspecific agonistic behavior in leopards (Steyn and Funston, 2006) and resulted in the death of the only collared female leopard in Tandoureh.

Table 4.3 A review of comparative intra-male home range overlap at different study sites, based on telemetry techniques. MCP=minimum convex polygon, KDE=Kernel density estimation and AKDE=auto-correlated Kernel density estimation.

Location/country	**Tracking method**	**Estimation method**	**Intra-male home range overlap (%)**	**Study**
Kruger National Park	VHF telemetry	MCP	21.5	(Bailey, 1993)
Lolldaiga Hills, Kenya	VHF telemetry	MCP, grid-cell and harmonic mean methods	0-9	(Mizutani and Jewell, 1998)
Kaeng Krachan National Park, Thailand	VHF telemetry	MCP	40	(Grassman, 1999)
Waterberg Plateau, Namibia	VHF telemetry	MCP	24 ± 13	(Marker and Dickman, 2005)
Bardia National Park, Nepal	VHF telemetry	KDE	7	(Odden and Wegge, 2005)
Phinda Game Reserve, South Africa	VHF & GPS telemetry	KDE	4.5 ± 1.5	(Fattebert et al., 2016)
Tandoureh National Park, Iran	GPS telemetry	AKDE	43.3 ± 5.9	This study

An obvious limitation of our study is the small sample size of GPS collared leopards, of which most were male (five out of six). Clearly the findings on a single female risk influence by individual idiosyncrasy (Börger et al., 2006). Nevertheless, besides Simcharoen et al. (2008) pioneering work (with eight collared leopards), our study is the most intensive study ever conducted on Asian leopards in terms of sample size and collaring period, reflecting the difficulty of working in the harsh landscapes in which Asian leopards persist.

4.5.3. Conclusion and synthesis

Home range, as described by Powell and Mitchell (2012), is “that part of an animal’s cognitive map of its environment that it chooses to keep updated”. We speculate that our findings support an ‘anchoring’ and ‘adjustment’ paradigm in the use of space. Anchoring and adjustment, are cognitive biases in the assessment of risk first described in humans by Tversky and Kahneman (1974). According to this psychological heuristic, when people assess the magnitude of a risk, they start with an implicitly suggested reference point (the "anchor") and make adjustments to it to reach their estimate.

We do not imply the same mechanisms underlying this heuristic in humans apply to leopards, not least as the concept of ‘rational choice’ has a different meaning in non-humans (Stanovich, 2013). If only in the form of a helpful analogy, the national park may be functioning as an ‘anchor’ for leopards (and probably many other animals) while they adjust their cognitive space use beyond the park boundaries (where, incidentally, they are not often associated with stock raiding). None of our collared leopards was killed by humans, whereas in the absence of properly managed protected areas, leopards can experience high rates of human-induced mortality in multi-use lands (Swanepoel et al., 2015; Williams et al., 2017).

Such anchoring and adjustment behavior supports the proposition that, in Asia’s rugged landscapes, a combination of land sparing and land sharing strategies at multiple spatiotemporal scales has the potential to ensure viability of leopards and other big cats. Properly-managed conservation areas (spared lands) are of paramount importance for securing high densities of large carnivores, insofar as they control poaching of carnivores and their prey species. Nonetheless, their space use outside-conservation areas must be managed through promoting the existence of carnivores in human-dominated landscapes (“land sharing”), with minimized levels of conflict with stock breeders.

With 25% of the global land surface area, mountain ecosystems support a wide range of ecosystem services and biodiversity (Körner, 2007). Climate change is expected to have a radical effect on biodiversity in mountainous areas (Thuiller et al., 2005), forcing northward and upward range shifts in many mammalian species (Hickling et al., 2006; Luo et al., 2015; Marino et al., 2011), including humans (Nogués-Bravo et al., 2007). Asian mountains can serve as climate refugia for big cats (Forrest et al., 2012; Li et al., 2016), despite the fact that only one third of their current extant range remains as suitable habitat in the next half century (Ebrahimi et al., 2017; Li et al., 2016). Land use change is the main driving factor for range losses in threatened mammalian carnivores (Di Minin et al., 2016). Conservation policy should clearly be proactive wherever possible for sparing montane refugia, preferably larger and better-connected areas, to anchor a high density of breeding nuclei of large cats in Asia’s rugged landscapes. Nonetheless, many montane protected areas are not large enough to meet extensive spatial requirements, high energy needs and hierarchical social interaction of big cats (Johansson et al., 2016). Therefore, bolstering the coexistence model (i.e. land sharing) is inevitable in order to support viability of both big cats and human communities, which are strongly dependent on reduced water resources in high altitudes. Future research might usefully explore the interaction between the land sharing and sparing, and how it can support both larger carnivore viability and human livelihoods, particularly in the context of montane landscapes.

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Chapter 5:

Persian leopard predation patterns and kill rates in the Iran-Turkmenistan borderland

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Persian leopard predation patterns and kill rates in the Iran-Turkmenistan borderland

5.1. Abstract

Describing predation patterns and especially estimating kill and consumption rates is essential for understanding the functional responses of predators. An understanding of the carrying capacity of the landscape, based on prey availability, also helps to formulate recovery plans for persecuted species. We studied the feeding behavior of the Persian leopard *(Panthera pardus saxicolor)* in Tandoureh National Park (355 km^2) in northeastern Iran, near the Turkmenistan border. Between September 2014 and May 2017, we collared and monitored six adult leopards (5 males and 1 female) using GPS-satellite Iridium collars. We investigated 310 clusters of fixes as likely to be kill sites. In total, 130 kills were identified to species, suggesting a mean kill rate of approximately 3.3 ± 0.3 (SE) kills/month per adult male leopard, which is higher than reported by most previous studies. The leopards varied considerably in the time they spent outside the national park; only one individual appeared to subsist mainly by stock raiding. The availability of medium-sized ungulates at adequate densities is likely to be important for future leopard conservation efforts. The management of problem individuals may also promote human coexistence with leopards with communities, even in prey-rich areas.

Key words: conflict, kill rate, Persian leopard, predation behavior, prey choice, satellite telemetry.

5.2. Introduction

Of the big cats, common leopards *(Panthera pardus)*, have the broadest dietary requirements (Sandom et al., 2018), and are widely perceived to be generalist predators (Martins et al., 2011; Shehzad et al., 2015). Linked to that, they have flexible habitat requirements (Gavashelishvili and Lukarevskiy, 2008). However, studies using fine-scale radio-tracking data have revealed that leopard populations can show some degree of specialization in their choice of prey (Radloff and du Toit, 2004; Stander et al., 1997) and hunting habitat (Balme et al., 2007).

The predatory behavior of leopards has been investigated across a range of habitat types using fecal analysis (see Stein and Hayssen, 2013), spoor tracking (Bothma and le Riche, 1982; Stander et al., 1997), direct sighting (Balme et al., 2007; Bothma and le Riche, 1982) and telemetry (see Stein et al., 2015). There is consequently a wealth of knowledge on leopard diets for most of their global range.

The rates at which prey are killed and consumed are less well understood. Effective management of predator–prey systems largely depends on reliable estimates of these metrics (Knopff et al., 2010). As well as informing predator-prey dynamics (Wegge et al., 2009), they are likely to indicate the extent of conflict with local stockholders (Farhadinia et al., 2014b; Ghoddousi et al., 2016) and also allow estimation of the nutritional carrying capacity of an area (Hayward et al., 2007; Jooste et al., 2013). Detailed knowledge of predation patterns can also reveal individual dietary specialists which are increasingly recognized as being widespread in predator populations (Lowrey et al., 2016). These attributes of predation are not straightforward to study. Problems with estimating components of predation in previous studies include tracking sessions that are too short (19 days; Odden and Wegge, 2009), inadequate sampling of kill sites (Martins et al., 2011), delays in identifying kill sites due to time lapsing before downloading data (Stein et al., 2015), and failure to verify kill sites identified from clusters of movement fixes (Rozhnov et al., 2015).

The substantial variation in previous estimates of kill rates, from 1.5 prey items per month (based on VHF radio-telemetry; Hamilton, 1981) up to 10 items per month (using spoor tracking; Bothma and le Riche, 1982) may reflect biological differences across the leopard's geographic range, methodological inadequacies, or both. To circumvent these problems, GPS telemetry has been recommended (Elbroch et al., 2017; Knopff et al., 2009). This approach requires that fix success, which is defined as the proportion of all scheduled fix attempts that are successful is > 45% (Knopff et al., 2009) and that predation sequences are continuously monitored in the field over long periods (Laundre, 2005).

The endangered Persian leopard *(P. p. saxicolor)* currently exists in only 16% of its former range in west and central Asia (Jacobson et al., 2016). Its prey has been documented to vary from rodents to domestic cattle and horses (Ghoddousi et al., 2016; E. Sharbafi et al., 2016; Taghdisi et al., 2013). However, rigorous quantification of predation patterns are sparse for leopards in Asian montane landscapes (Wegge et al., 2009). This is especially pertinent for the Persian subspecies whose natural prey is being depleted across west and central Asia (Farhadinia et al., 2014b).

In this paper, we used GPS data to address four objectives concerning the predation patterns and foraging behavior of Persian leopards, the apex predator in Tandoureh National Park (NP) along the Iran-Turkmenistan borderland. First, we explored leopard prey choice and how it is affected by spatiotemporal factors. Second, we quantified kill rates (number of prey per individual per month) and then converted kill rates to estimates of consumption rates. This information is particularly important where prey species are trophy hunted, as is the case around Tandoureh NP and other mountainous reserves. We also explored inter-kill intervals and handling times, hypothesizing that larger prey body mass would require greater handling time and be followed by longer inter-kill intervals compared with smaller prey items.

5.3. Materials and Methods

5.3.1. Ethics statement

Capture and handling of leopards followed the guidelines of the American Society of Mammologists (Sikes, 2016). The Iranian Department of Environment reviewed and approved all sampling, trapping and handling procedures (research permit number 93/16270). The trapping and handling protocol was also approved by University of Oxford’s Ethical Review Committee (BMS-ERC-160614).

5.3.2. Study Area

Tandoureh NP in north-eastern Iran (ca. 20 km from the Turkmenistan border) has been protected since 1968 and covers 355 km^2. It is characterized by mountains populated with wormwood *(Artemisia sp.)* and scattered juniper trees *(Juniperus sp.)* Elevation ranges from 1,000 to 2,600 m. Human settlements, most of which are associated with sheep and goat herds border the park. Potential wild ungulate prey for leopards is limited to urial *(Ovis orientalis),* bezoar goat *(Capra aegagrus),* and wild pig *(Sus scrofa)* which are all subject to occasional trophy hunting in the mountainous terrain surrounding the national park.

The availability of leopard prey in Tandoureh NP is affected by the national park boundaries. Wild bovids occur almost entirely inside the park, wild pigs occur throughout but at reduced densities outside the park, and domestic animals such as sheep *(Ovis aries)*, goat *(Capra hircus)* and domestic dog *(Canis familiaris)* are found almost exclusively outside the park across multi-use lands.

5.3.3. Leopard capturing and collaring

We captured leopards with Aldrich foot-snares modified extensively to reduce the chance of injury (Frank et al., 2003). Traps were fitted with remotely monitored VHF transmitters (Wildlife Materials, Inc., Illinois, USA) emitting signals every 1-2 hours (see Farhadinia et al. 2017 for more details). As leopards are known to respond to baits (du Preez et al., 2014), a wild pig carcass was used as bait, normally hanging from a tree or rock. Traps were also deployed along trails leading to the baits. In summer, we deployed traps along trails leading to water sources, sometimes without bait (see Farhadinia et al. 2017 for more details).

We immobilized leopards using a combination of ketamine 10% (Alfasan, Nederland BV) 2 mg/kg, medetomidine HCl 20 mg/ml (Kyron Laboratories (Pety) Ltd., Johannesburg, South Africa) 30 µg/kg and butorphanol 0.2 mg/kg (Torbugesic®, Fort Dodge Animal Health Fort Dodge Animal Health, Iowa 50501 USA) delivered intramuscularly with a dart gun (Daninject, Denmark) using a 1.5 ml dart.

We used GPS collars with Iridium download (LOTEK Engineering Ltd., Newmarket, ON, Canada). Each collar incorporated a drop-off buckle with a timer set to 52 weeks post-deployment. Collars weighed 640 g, equivalent to less than 1-2% of leopard body mass. Each animal's age was estimated based on dentition (Stander, 1997). Anesthesia lasted for between 44 and 60 minutes, followed by reversal using atipamazole (3 times the medetomidine dosage) and nantroxan (the doses equal to butorphanol), injected intramuscularly.

5.3.4. Cluster investigation

We followed Knopff et al. (2009) who recommended recording fixes every 3 hours to enable the identification of spatially aggregated GPS points, or clusters, while ensuring the survival of transmitters batteries for at least one year. This was based on a study of cougars *(Puma concolor)* feeding on prey > 8kg. Collars were programmed with a 'virtual fence' option triggering an increased fix rate, to hourly, when leopards left the park. This enabled us to detect kills reliably outside the national park where prey generally have a smaller body mass (i.e. domestic animals) and scavengers, such as herding and stray dogs, golden jackal *(Canis aureus),* and striped hyena *(Hyaena hyaena)* are more abundant.

Initially, clusters were defined as ≥ 2 locations within 100 m of each other and with the earliest and latest location separated by less than 12 hours (Johansson et al., 2015; Knopff et al., 2009; Martins et al., 2011). Handling time and cluster fidelity are two predictors of kill clusters (Knopff et al., 2009; Martins et al., 2011), but clusters using only daytime locations yielded remains of prey at only two locations (two bezoar goats in 36 visits).

To determine the timing of feeding, we deployed Bushnell Trophy Cam camera traps (Bushnell Outdoor Products, Overland Park, KS, USA) at fresh kills or baits (n = 14) in northeastern Iran. This revealed that both male and female leopards remained on kills overnight (6 urials, 2 wild pigs and 6 dogs), with the exception of one occasion when a female and cub did not return to their kill after deployment of the camera trap. Our observations supported the conclusion that leopards predominantly feed at night (Stander et al., 1997). As a result, in accordance with

Johansson et al. (2015), we considered daytime clusters to be rest sites. Therefore, GPS clusters where leopards remained overnight (between 6 PM and 6 AM) within a radius of 200 meters were investigated for possible kill remains. Nonetheless, we acknowledge that we may have missed small prey, especially if they were consumed during daylight.

We identified clusters visually using Google Earth 5 (Google Corporation, 2009) and a web-based map-system for displaying telemetry data (webservice.lotek.com) in a 6-8 day time-frame. Distances between each consecutive GPS locations were calculated. Candidate GPS cluster locations were then uploaded on a hand-held GPS device (Garmin GPS62S, Garmin International, Olathe, KS, USA) to enable ground crews of ≥ 2 people to systematically search within a radius of at least 100 meters from each cluster location for at least 20 minutes, following (Knopff et al., 2009).

Prey remains were thoroughly investigated to identify prey species, and where this was uncertain hair samples were taken for lab investigation based on a reference hair collection (Rezaei, 2014). We used horn size and annual growth rings to assign sex and age to bezoar goat and urial. Additionally, long white hairs of adult urial rams were diagnostic of sex. Age categories were defined as adult (> 4 years old), young (> 1 and 4 ≥ years old) and lamb (1 ≥ year old).

Cluster locations were visited over 87 days between September 2014 and May 2017. Twelve were discarded as not being accessible because of extreme weather conditions at high elevations, leaving a total of 310 locations which were investigated as candidate clusters (Table 5.1). Cluster sites were visited only after at least two days had elapsed since the leopard's last location at the site. In the dry montane landscape, hair, horns, bone pieces, etc. can be detected several months after a kill (Johansson et al., 2015). We included only kills found within 120 days of the identification of a cluster to estimate the consumption rate. We split monitoring time into multiple sampling periods in which we visited every cluster to avoid bias caused by inclusion of non-investigated clusters.

5.3.5. Prey characteristics

Prey species were allocated to two size categories. The “small” prey (< 15 kg) included red fox *(Vulpes vulpes)*, Indian crested porcupine *(Hystrix indica)* and birds. The “medium” prey (≥ 15 kg) category included urial, bezoar goat, wild pig, domestic sheep and domestic dog. Young wild ungulates and domestic animals were also included in medium-sized prey.

In Tandoureh, prey availability differs markedly on either side of the national park boundary. Thus, to understand patterns of predation on wild (inside the NP) versus domestic medium-sized prey (outside NP), we calculated the time (days) each leopard spent either inside or outside the national park as a proxy for livestock versus wild prey spatiotemporal availability.

For those medium-size prey for which sex was determined (i.e. urial and bezoar goat), contingency-table-based chi-square tests were also used to explore sex-biased selection. Sex ratios were based on observations of seasonal prey count surveys along transects laid within the national park, usually along ridgelines or valley bottoms. The time, location, sex/age structure (using 12×42 binoculars and 20-60 telescope) of all urial and bezoar goat sightings were recorded. Transects, varying in length between 5 and 26 km, were laid at least 3 km apart in order to avoid double-counting. In total, 247 km of transects were surveyed between August 2013 and March 2015. Although there was partial temporal overlap between our prey survey and cluster investigations (September 2014– May 2017), there was no evidence of any major variation in the prey populations during this period. Therefore, we are confident that the estimates of ungulate population demographics can be compared with our exploration of predation based on the location of clusters.

To minimize misidentification of different age/sex groups, we excluded all sightings further than 700 m from the center line of each transect. Distances were measured using a Nikon Monarch Gold Laser1200 Long Range Rangefinder (Nikon Inc., NY, USA). We improved the accuracy of urial and bezoar goat detection at sex/age level using images taken with a digital zoom cameras (Nikon P500 36X optical zoom or Fuji Finepix S1 50X optical zoom). For better identification of age/classes based on size and shape of horns, urial and bezoar goat images were investigated on a computer and thus the proportion of individuals within each sex/age class category was calculated. We then used Fisher's exact test to test if prey age was independent of leopard age. Each age was defined as either young (< 4) or adult (≥ 4) for leopards.

To explore prey choice, we used mixed effects cumulative link models for the analysis of nominal responses. Multinomial models were fitted using the ‘Mixcat’ package in R (Papageorgiou and Hinde, 2012). Each prey type was treated as a nominal response variable. Predictor variables included season (four seasons), the time since leaving the last kill (last fix at radius of 200 meters of where the prey was found) as well as last meal size (kg). To account for individual variability in prey choice, leopard ID was assigned as a random effect which was about the minimum number (six levels) required for obtaining reliable estimates for random effects terms (Bates, 2010).

Seasons were defined as March to May = spring, June to August = summer, September to November = autumn, December to February = winter. The significance of terms in the final model was assessed using log-likelihood ratio tests for comparing the goodness of fit between models.

5.3.6. Kill and consumption rates

Topography and animal behavior can influence the performance of GPS telemetry collars, including fix success rates which results in missing data (Cain III et al., 2005). Although our overall fix rate was high (mean 85.0% ± SE 7.6), we removed kill intervals containing overnight fix failures (n = 9) before analysis (either unsuccessful or non-retrieved fixes). Prey scavenged by leopards (n = 9), small prey (n = 9) and those found after four months (n = 21) were also excluded from kill rate calculations. In total, 91 medium-sized prey remains were used to estimate kill rates for medium size prey.

We used a ratio estimator (Hebblewhite et al., 2003) which truncates the denominator to the period between the first and last kills of each sampling period. We used total monitoring time (as a cumulative of all sampling periods) as the denominator for rate estimation and both number of kills and kilograms of prey as numerators, yielding frequency (kills/month) and biomass (kg/day) metrics for each individual (Knopff et al., 2010; Miller et al., 2013). We acknowledge that we estimated the potential maximum consumption rate of detected kills, because we assume that all edible parts of each kill are consumed by the leopards.

In order to calculate biomass consumed by the leopards, we assigned approximate live weights to prey, using values from the literature or from measurements made in northeastern Iran. Weight estimates for adult female and young male urials were obtained from Valdez et al. (1977) while weight measurements were collated for adult male urial (n = 10), all age/sex categories of bezoar goat (n = 11), red fox (n = 8), domestic sheep (n = 3), and domestic dog (n = 2), all from intact carcasses found in the field. We were not able to identify the sex of the wild pigs and porcupines, so species-level average body mass were obtained from Goshtasb (2001) and Mori and Lovari (2014), respectively.

We weighed the horns of male urials and bezoar goats (both adult and young) and subtracted their weight from mean body mass. Then, we used regression to calculate the non-consumable portion of a prey carcass typical for big cats (Fàbregas et al., 2017). We concluded that 22% was non-edible for bezoar goat and urial, 25% for wild pig, 21% for domestic dog, 23% for domestic sheep, 16% for Indian crested porcupine and 12% for red fox.

We found no evidence for non-trivial rates of domestic animal raiding around Tandoureh using our questionnaire surveys (Farhadinia et al., 2017). Thus, in order to calculate monthly predation rates for each prey species, we omitted data from leopard M1, because of his unusually high rate of depredation on domestic stock.

5.3.7. Patterns of hunting behavior

Leopards are known to drag their kills to a concealed location before consumption; recorded an average distance of 140 m from the kill site (Stander et al., 1997). Thus in order to calculate time of day when the leopard hunted, denoted as ‘predation time’, we estimated the earliest time within 200 m of each kill cluster centroid. ‘Handling time’, defined as amount of time a leopard spent on a kill was calculated as the time difference between first and last times within this radius (Knopff et al., 2010). If the leopard wandered outside the kill area, that time was subtracted from the handling time. We also estimated ‘inter-kill interval’ as the time period between two consecutive predation times. Finally, ‘search time’ was calculated as the inter-kill interval with handling time subtracted , i.e. the time elapsed since leaving the last meal and making the next kill (Hebblewhite et al., 2003; Knopff et al., 2010).

We developed a generalized linear model to relate a handling time response to kill location inside/outside Tandoureh) and last prey weight. Also, another set of generalized linear models were constructed to explore how season, location and last prey weight (kg) were associated with a response defined as inter-kill interval. Finally, we modelled the effect of season on kill rate and consumption rate of leopards. Leopard ID was treated as blocking factor in all modeling attempts. Response variables were square root transformed, because model diagnostics were more compatible with distributional assumptions comparing to non-transformed and log-transformed data. Model selection for inter-kill interval was performed using corrected AIC (AICc) criteria, using ‘AICcmodavg’ package (Mazerolle, 2011). All means are reported with standard errors. We conducted all analyses in *R* (R Development Core Team, 2013).

5.4. Results

Between September 2014 and May 2017, six leopards (5 males and 1 female) were collared and monitored in and around Tandoureh, comprising 4 adults and 2 young individuals (Fig. 5.1). Their kills were monitored for 1554 leopard-days (mean = 259.0 ± 52.6 days/leopard, Table 5.1). The leopards varied considerably in the time spent outside the national park within multi-use areas (in total, 17.9 ± 7.3 % of tracking days overall, varying between 0.0 and 43.8% for different individuals). Field signs confirmed that the leopards scavenged 9 times (varying between 0 and four events among individuals), mostly at baits we deployed for capturing and collaring operations (Table 5.1). Four clusters were excluded because of mismatches between dates of the cluster and the age of the kill found there.

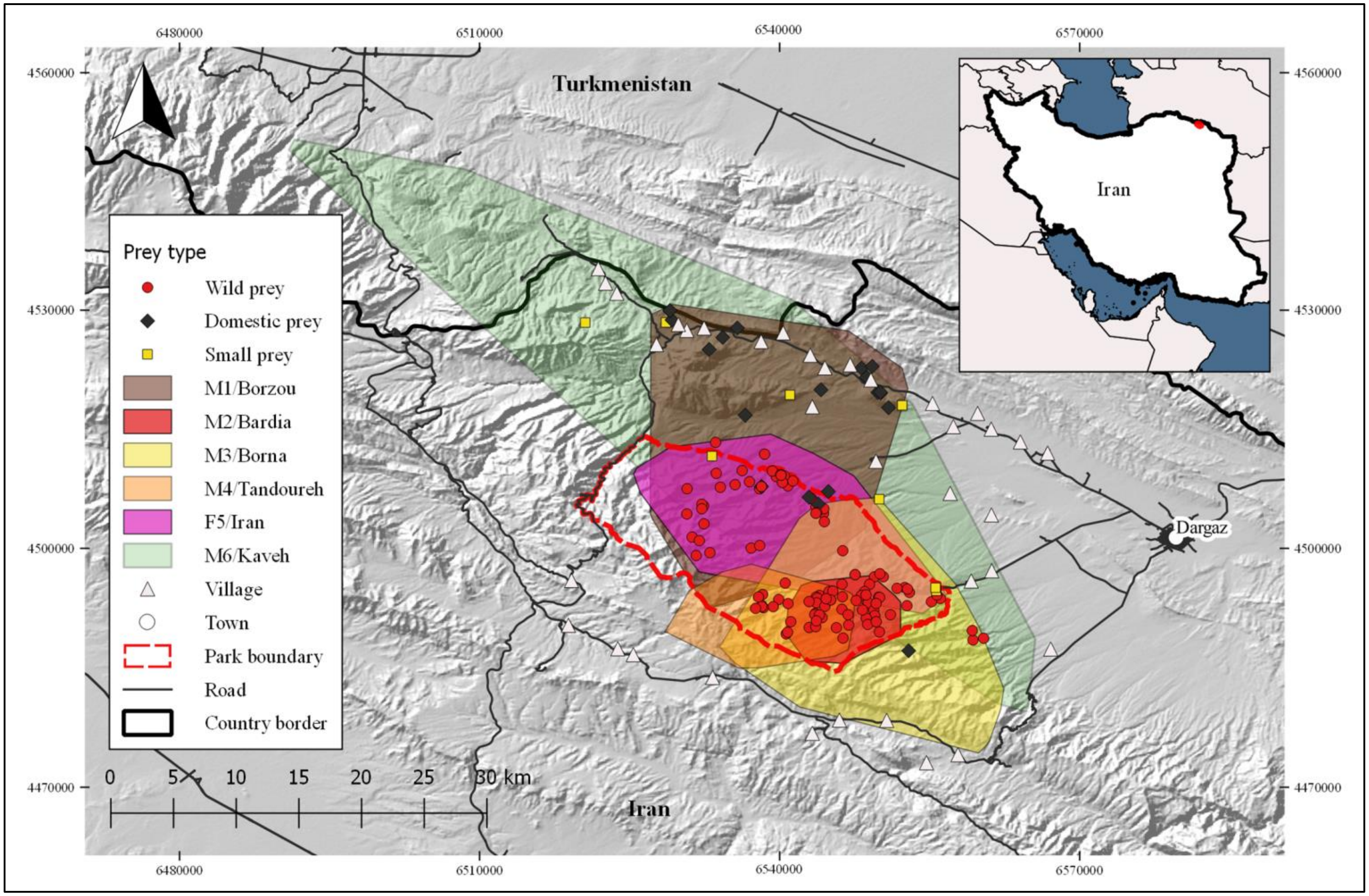

Fig 5.1 Distribution of all leopard kill sites located inside and outside Tandoureh National Park from 2014 to 2017. Each polygon represents a collared leopard’s 100% minimum convex polygon. Domestic prey were confined to areas outside the national park. In contrast, wild ungulates were predominantly killed within the park boundaries.

Table 5.1 Summary of data used for estimating kill rates and potential consumption rate of Persian leopard in Tandoureh National Park, from 2014 to 2017.

Leopard ID	**Sex/Age**	**No. collaring days (days outside NP)**	**No. clusters searched**	**No. Scavenges**	**No. Kills**	**Prey species**										**Kill rate (kill/month)**[d]	**Consumption rate (kg/day)**
						Urial	**Bezoar goat**	**Wild pig**	**Sheep**	**Dog**	**Porcupine**	**Fox**	**Raptor**	**Chukar**	**Pigeon**		
M1	M/+10	368 (161)	106	4	43	17	3	1	4	14	1	1	0	1	1	3.7	4.4
M2	M/7-10	362 (0)	70	1	28	5	23	0	0	0	0	0	0	0	0	3.4	4.5
M3	M/4-6	364 (49)	57	0	21	11	5	2	1	0	1	0	1	0	0	2.2	2.9
M4	M/7-10	228 (5)[a]	29	0	20	13	6	0	0	0	0	1	0	0	0	5.1	7.1
F5	F/2-3	54 (4)[b]	17	3	2	2	0	0	0	0	0	0	0	0	0	NA	NA
M6	M/3-4	178 (59) [c]	31	1	16	9	1	3	0	1	0	1	1	0	0	4.1	5.5
Total		1554 (278)	310	9	130	57	38	6	5	15	2	3	2	1	1		

[a] The collar stopped functioning on 1 April 2017, probably due to battery failure. The animal was spotted again on 3 June 2017 with the collar.

[b] Killed by a larger conspecific on 29.1.2016.

[c] M6 was collared for 326 days, but only 178 days spent within Iranian territory where we were able to closely monitor predation behavior of the animal. On 29 February 2016, he left Iran for Turkmenistan.

[d] 91 medium-sized prey remains were used to estimate kill rate, after exclusion of prey scavenged by leopards (n=9), small prey (n=9) and those found after four months (n=21).

5.4.1. Prey characteristics

We found 130 prey items of 10 species which we assigned to three categories (Fig. 5.1 and Table 5.1): wild ungulates (urial, bezoar goat and wild pig), domestic animals (dog, sheep) and small animals, such as Indian crested porcupine, red fox, raptors, pigeon *(Columba livia)* and chukar partridge *(Alectoris chukar)*. Domestic prey were killed exclusively outside the national park, whereas 96.6% of all wild ungulate kills were within the park (Fig. 5.1). Non-domestic kills outside the park were urial (n=3), wild pig (n=2), small mammals, and birds (Fig. 5.1). There was no evidence that prey type was influenced by season, the weight of the last prey item or the search time since last kill (Table 5.2).

The majority of prey items were medium-sized species, with small animals comprising only a small minority (93.3% vs. 6.7%, Table 5.3). Although wild prey dominated leopard diet (81.5% vs. 18.5% domestic animals; Table 5.3), there was no evidence that the frequency of prey types (wild vs. domestic animals) was out of proportion with the amount of time that leopards spent inside or outside the national park ($X^2_1 = 0.01$, $P = 0.92$). Amongst individual leopards, there was a strong positive correlation between the proportion of time each spent outside the national park and the percentage of kills each made of domestic stock ($r_s = 2.06$, $P = 0.005$). Although domestic animals were not detected in three individual’s investigated clusters, they comprised 46.2% of medium-sized kills made by M1 (old male; Table 5.1).

A total of 176 groups of urial and bezoar goat were detected, but 31 were excluded as their distance did not allow us to identify their age/sex composition. Consequently, 1875 individual urials (100 detections) and 918 bezoar goats (45 detections) were recorded (Table 5.S1). In urials, 90.0% of young males were detected in maternal groups whereas 69.9% of young male bezoar goats were spotted in such mixed groups. A grand mean sex-ratio of 40.7 (± 7.5) adult rams was calculated per 100 urial ewes whereas there were as 40.6 (± 0.4) adult males per 100 female bezoar goats. Of 95 kills of urial and bezoar goat (73.1% of the total, Table 5.1), 87.1% were males versus 12.9% female, 80.8% were adult versus 19.2% young or lamb. The observed male biased predation was statistically significant for both urial and bezoar goat ($X^2_1 = 54.0$, $P < 0.005$ and $X^2_1 = 72.4$, $P < 0.005$, respectively). Young leopards killed both young and adult prey equally (50.0% vs. 50.0%), whereas adult leopards killed a preponderance of adult prey (88.1% vs. 11.9%; Fisher's exact test, $P = 0.003$). Adult male urials and bezoar goats killed by the leopards were on average 7.1 ± 0.4 and 6.9 ± 0.3 years old. Four of six wild pig remains were young adults (< 2 years).

Table 5.2 Result of sequential likelihood ratio tests of multinomial models testing the effect of season, last prey weight or search time since last kill on prey type. LR stat. denotes to likelihood ratio statistic (difference of residual deviance).

Model No.	Explanatory variables	Test	DF	LR stat.	*P*
1	Null	NA	NA	256.9	NA
2	Season	2 vs. 1	3	255.9	0.79
3	Search time	3 vs. 1	1	256.7	0.60
4	Last meal weight	4 vs. 1	1	258.5	0.21

5.4.2. Kill and consumption rates

The only collared female leopard (F5) consumed 127.5 kg of edible prey during 54 tracking days, amounting to 2.4 kg/day. For males, we estimated a rate of 3.3 ± 0.3 kills/month, equal to a daily rate of 0.11 ± 0.01 kill. Kill rates varied substantially among individuals (range 2.2-4.1 medium size prey/month and 2.9-5.5 kg/day, Table 5.1). Each adult male leopard consumed an average of 129.6 ± 2.0 kg/month, i.e. 4.3 ± 0.1 kg daily (Table 5.1), equivalent to an average of 40.0 ± 3.9 medium size prey per annum (1554.8 ± 24.6 kg of medium size edible biomass).

There was no evidence for seasonal variation in either kill rate ($F_{3,\,8} = 1.01$, $P = 0.44$, Fig. 5.2a) or consumption rate ($F_{3,\,8} = 1.58$, $P = 0.27$, Fig. 5.2b).

On three occasions a cluster revealed two prey (cases 1 and 2 for leopard M1: wild pig and urial, dog and domestic sheep, case 3 for M4: urial and red fox). Additionally, M1 killed two dogs at the same time, of which he carried off only one.

5.4.3. Patterns of hunting behavior

The leopards spent an average of 51.9 ± 2.9 hours at each kill. At 30.1% of the kills leopards spent some time moving beyond the feeding area (3.0 ± 1.0 hours), to which they intermittently returned. Handling time (time spent at a kill) was not correlated with prey body mass ($r^2 = 0.38$, $P = 0.71$). There was no evidence that the prey weight ($F_{1,\,96} = 0.01$, $P = 0.94$) or whether the kill occurred inside or outside the park ($F_{1,\,95} = 0.61$, $P = 0.43$) affected handling time in a cluster.

There was no evidence that the inter-kill intervals were usefully predicted by any of the modelled variables (the best performing model was the null, including only leopard ID; Table 5.4). Summer inter-kill intervals were shortest (6.7. ± 1.2 days; $F_{3,\,90} = 1.82$, $P = 0.15$, Fig. 5.2c). In total, 50.0% (n = 11) of summer kills within the national park were within a kilometer distance of the nearest water resource.

Table 5.3 Prey species located at Persian leopard cluster kills sites (2014-2017) in Tandoureh National Park.

Prey species	**No. kills located**	**% total kills**	**% biomass**	**Kills/month (SE)**
Urial sheep *(Ovis orientalis)*	57	43.8	43.5	2.08 (0.75)
Bezoar goat *(Capra aegagrus)*	38	29.2	36.2	1.14 (0.51)
Wild pig *(Sus scrofa)*	6	4.6	7.8	0.24 (0.16)
Domestic sheep *(Ovis aries)*	5	3.8	4.5	0.03 (0.03)
Domestic dog *(Canis familiaris)*	15	11.5	8.0	0.09 (0.09)
Indian crested porcupine *(Hystrix indica)*	2	1.5		
Red fox *(Vulpes vulpes)*	3	2.3		
Birds	4	3.1		
Total	130			

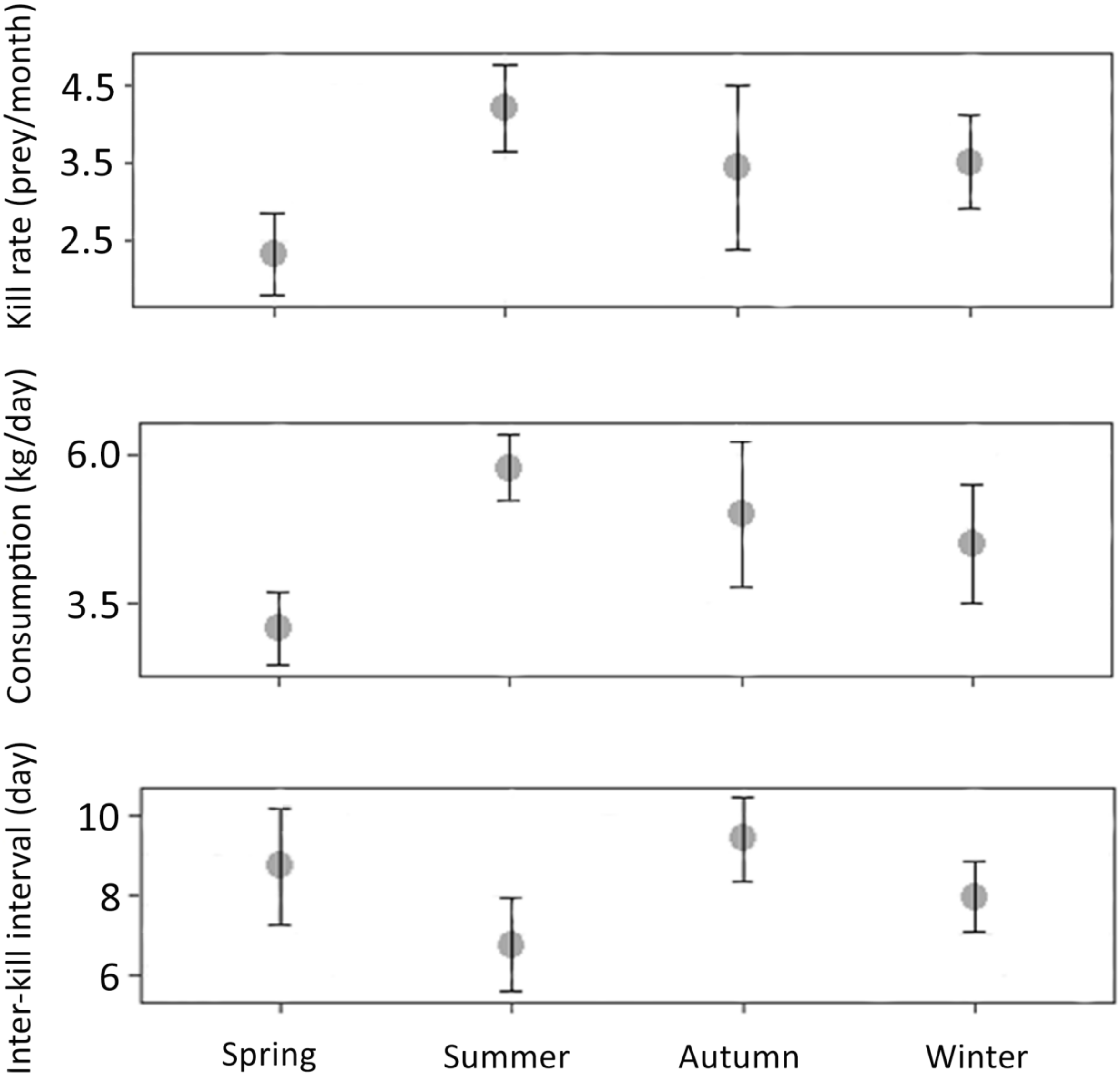

Fig 5.2 Seasonal differences in (a) kill rate (prey/month), (b) consumption rate (kg/day) and (c) inter-kill interval (day) of Persian leopards *(Panthera pardus saxicolor)* in Tandoureh National Park, Iran (2014–2017). Error bars represent standard errors.

Table 5.4 Results of linear mixed model fit with random slope by REML (residual maximum likelihood) criterion to explore effect of different predictors on inter-kill intervals (IKI) of the leopards in Tandoureh National Park from 2014 to 2017. IKI was square rooted to meet normality and variance equality assumptions.

Model number	Model	K	AICc	∆AICc	AICc Weight	Cum. AICc Weight
1	IKI ~ LeopardID	1	205.1	0	0.40	0.40
2	IKI ~ LeopardID + Last Prey Weight	2	205.9	0.8	0.27	0.66
3	IKI ~ LeopardID + Location	2	207.4	2.3	0.12	0.79
4	IKI ~ LeopardID + Last Prey Weight + Location	3	208.3	3.2	0.08	0.87
5	IKI ~ LeopardID + Season	2	208.5	3.4	0.07	0.94
6	IKI ~ LeopardID + Last Prey Weight + Season	3	210.1	5.0	0.03	0.97
7	IKI ~ LeopardID + Location + Season	3	211.0	5.9	0.02	0.99
8	IKI ~ LeopardID + Last Prey Weight + Location + Season	4	212.8	7.7	0.01	1.00

5.5. Discussion

We observed a kill rate higher than most previously published estimates for leopards. Wild ungulates were the principal prey. Leopards showed age-dependent selectivity for certain prey age/sex groups. A single individual relied mainly on domestic stock, while others predominantly killed wild ungulates.

5.5.1. Prey characteristics

Young leopards preyed mainly on young wild ungulates whereas adult individuals predominantly hunted adult ungulates. Prey body mass, a key predictor of prey selection, may explain this selective foraging in different ages (Elbroch et al., 2017; Knopff et al., 2010; Radloff and du Toit, 2004).

Leopard populations are known to show some degree of specialization in their choice of prey (Stander et al., 1997) or hunting habitat (Balme et al., 2007). Our data revealed selective hunting of adult male (> 4 years) urials and bezoar goats. There are few previous observations of sexual selectivity by leopards. Owen-Smith (2008) observed an excess of female impala *(Aepyceros melampus)* in leopard diet, but males are more commonly targeted (Bailey, 1993; Karanth and Sunquist, 1995). Anti-predator vigilance, which is more common in females and kids among mountainous wild sheep and goats (Cransac et al., 1998; Grignolio et al., 2007) is a possible explanation for apparent male-selective predation by Persian leopards. Similarly, the majority of young males of both principal prey species, i.e. urial and bezoar goat observed in Tandoureh were accompanied by maternal groups, which may provide some defense against predators. Predator populations may be an aggregate of individual dietary specialists (Bolnick et al., 2002), dietary variation possibly reflects differences among individuals in foraging behavior rather than prey availability (Lowrey et al., 2016). Future research should therefore address individual variation in predatory behavior.

Previous studies on Persian leopard diet in northeastern Iran based on fecal analysis (Farhadinia et al., 2014b; Ghoddousi et al., 2016; E Sharbafi et al., 2016; Taghdisi et al., 2013) have shown consistent dominance of wild ungulates in the diet, estimated to constitute 80-95% of consumed biomass. We acknowledge that our measure of spatial availability of domestic animals could have benefited from incorporating the density of domestic versus wild prey. Nonetheless, the taking of domestic animals in Tandoureh was closely associated with the amount of time individuals spent in multi-use areas, i.e. outside the NP. Therefore dietary analysis based on fecal sampling within livestock free national parks might underestimate stock raiding by leopard individuals which generally happens outside the national parks. The minor role for domestic stock consumption (5-20%) recorded in previous studies may also be an underestimate, not reflecting stock predation and defecation in excursions beyond the national park boundary. Our data showed that even though most individuals had access to domestic stock when making periodic excursions in human-dominated landscapes outside the national park, only one leopard (the old male M1) regularly targeted the stock of local communities.

Our findings have some limitations. First, they are based on a limited sample of GPS collared leopards, and mostly males. Second, our data were restricted to larger prey items associated with clusters of fixes. All small prey items were detected along the individual Tabdoureh’s boundary or outside (Fig. 5.1), where the ‘virtual fence’ technology shifted the fix interval from every three hours to hourly. This will have reduced the likelihood of detecting smaller prey items inside Tandoureh. In contrast, our variable fix interval was unlikely to affect identification of medium-sized prey because the leopards spent on average two days at a kill, clearly long enough to detect a cluster. We could not assess the extent of predation on small bodied prey (rodents, lagomorphs and birds) that are unlikely to generate GPS clusters with our fix programming inside Tandoureh (Knopff et al., 2010). However, various fecal analyses revealed negligible consumption of small animals in northeastern Iran (Ghoddousi et al., 2016; Taghdisi et al., 2013). Third, young wild ungulates, particularly lambs (≤ 1) are likely to have been underrepresented in our kill data, because their small body size and delicate skeletons make them easy to consume rapidly, such that no cluster of fixes results. Large cats often show a seasonal shift to neonates after ungulate birth pulses (Knopff et al., 2010; Owen-Smith, 2008). To explore the seasonal shift hypothesis, future studies would need to use shorter intervals (≤ 1 hour).

5.5.2. Kill and consumption rates

Our kill rate estimate was higher than reported in most previous studies (Table 5). This is likely to be due to both methodological and ecological factors. Leopards vary markedly in size across their range. The Persian leopard is one of the largest subspecies (Stein and Hayssen, 2013), adult males weigh on average 65.8 kg (Farhadinia et al., 2014a). Higher mass-related energetic requirements may therefore at least partly explain the higher consumption rate (Carbone et al., 1999).

The majority of previous estimates (Table 5) are based on studies with limitations of some kind. In some the leopards were tracked for relatively short periods (Odden and Wegge, 2009); in others, small to large prey were included in the calculation (Bothma and le Riche, 1982), or some potential kills were neglected (Martins et al., 2011; Rozhnov et al., 2015). Similarly, relatively high kill rates were reported for Amur tiger *(P. tigris altaica)* (Miller et al., 2013) and cougar (Knopff et al., 2010) when GPS clusters were used compared with estimates derived from other techniques.

Our data revealed that estimates of kill rate varied almost 2.5-fold among individual leopards. Similar individual variation has been reported from other cats, such as jaguars *(P. onca)* (Cavalcanti and Gese, 2010), cougar (Knopff et al., 2010) and tiger (Miller et al., 2013). The lowest kill rate (by an adult male, M3) was associated with a home range in the eastern margins of Tandoureh where prey density was thought to be low.

5.5.3. Patterns of hunting behavior

Large felids tend to stay longer on larger kills where wider ranges of prey sizes are available (Cavalcanti and Gese, 2010; Knopff et al., 2010; Miller et al., 2013). The amount of time a leopard spent on a kill was not associated with either body mass of the prey or location (where inside or outside the NP). The limited range of prey size in Tandoureh (medium-sized mammals) may account for the lack of variation in handling time. Likewise, assigning kills to either inside or outside the NP may have not captured the necessary spatial scale at which distinct behaviors are affected by human interference outside the national park (Wilmers et al., 2013). We did observe that all kills made outside the NP were dragged into nearby rocky cliffs and mountains, a behavioral response to avoid human interference. Therefore the available landscape heterogeneity in these multi-use landscapes may provide sufficient cover for leopards to avoid the fear induced by humans (Smith et al., 2015).

Our data showed no evidence for seasonal differences in kill rate and predation patterns in Tandoureh. In contrast, seasonal predation pattern is widely reported in big cats, explained by migratory prey species, synchronized birth pulse in prey and changes in environmental situations or predator energetic requirements (Allen et al., 2014; Cavalcanti and Gese, 2010; Knopff et al., 2010; Miller et al., 2013). In Tandoureh, the ranging patterns of the principal prey species, i.e. urial and bezoar goat are mainly dictated by the national park boundary. Pastures outside the national park are occupied by herds of livestock with limited water availability. In the north, the park is surrounded by open farmlands while two roads delineate the park perimeter to the west and east. Seasonal migration would not therefore be likely to benefit wild ungulates.

The shortest inter-kill intervals were observed in summer. There are two possible explanations. First, in summer there are abundant vulnerable juvenile ungulates just after their birth pulse in spring (Laundre, 2008). No predated lambs were, however, found in summer. Alternatively, the shorter observed intervals in summer for medium sized prey could be explained by the ease of hunting ungulates around scarce water resources; half of summer kills were found near water resources.

5.5.4. Management implications

Our observations suggest, albeit tentatively given the low number of individuals observed, that domestic animals, even in areas where wild prey is abundant, can comprise a substantial part of leopard diets. It is also clear that stock raiding behavior may be confined to problem individuals rather than being a characteristic of the entire population. In general, problem individuals can be responsible for a disproportionate impact on human interests, such as health, culture, wellbeing, and economics (Swan et al., 2017). Selective management targeting specific individuals can therefore be effective in conflict mitigation (Linnell et al., 1999). Hitherto, translocation of problem leopards has often been favoured – the effectiveness of this is not well established (Odden et al., 2014; Weilenmann et al., 2011; Weise et al., 2015). As such, any attempt should be conducted under validated guidelines (Weise et al., 2015).

While leopards tend to be opportunistic predators, they do show a level of prey selectivity which should not be neglected where their prey species are exploited, for example by trophy hunting. Both leopards and trophy hunters select large ungulate males which can contribute to the skewed sex ratio towards females. If both trophy hunting of prey and leopard existence are to be sustainable, the number and spatial configuration of trophy quotas should be based on a sound evidence base - prey numbers should therefore be monitored.

Finally, our findings provide an empirical baseline for predicting the prey requirements of leopards and functional response of leopards which couples their intake rate to prey density. We also highlight the need for protecting prey population as a key component of leopard conservation. As change in land use is the main threat for the ranges of threatened mammalian carnivores (Di Minin et al., 2016), we recommend that future studies focus on understanding the energetic requirements of leopards in areas where they increasingly share the landscape with human communities.

Table 5.5 A review of comparative kill rates and food consumption rates of leopards at different study sites, based on different monitoring techniques.

		Kill rates (kill/month ± SE)			Consumption rates (kg/day ± SE)			
Habitat and location	**Method**	**Male**	**Female**	**Female with cub**	**Male**	**Female**	**Female with cub**	**Study**
Semi-arid savannah, South Africa	Spoor tracking	9.1		20	3.5		4.9	(Bothma and le Riche, 1982)
Woodland savannah South Africa	VHF collar	4.2	4			4.4–4.7		(Bailey, 1993)
Tsavo National Park, Kenya	VHF collar	1.5						(Hamilton, 1981)
Meru National Park, Kenya	VHF collar	2.3						(Hamilton, 1981)
Semi-arid savannah, Namibia	VHF collar and spoor tracking	3.1-3.3	1.6	2.4-2.5	3.1–3.3	1.6±0.5	2.6±0.5	(Stander et al., 1997)
Temperate mountains, Russian Far East	GPS Argos collar[a]		2.4					(Rozhnov et al., 2015)
Subtropical forest, Nepal	VHF collar			2.8	4.3±1.1		4.0±0.3	(Odden and Wegge, 2009)
Woodland savannah, Botswana	GPS collar	3.3	3.4	3.0				(Stein et al., 2015)
Open rocky mountain, South Africa	GPS collar[b]	2.3	2.0					(Martins et al., 2011)
Steppe mountain, Iran	Iridium-GPS collar	3.3±0.3			4.3±0.1			This study

[a] Potential kills were estimated based on cluster of GPS fixes, but were not visited in the field.

[b] Candidate clusters were partially verified in the field.

5.6. References

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Table 5.S1 Details of urial and bezoar goat detected during prey surveys between August 2013 and March 2015 in Tandoureh National Park, northeastern Iran. Grand means of all sex ratios were calculated to assess sex-biased selectivity in predation.

Species	**Date**	**Effort (km)**	**Number of detections**	**Total number of individuals**	**Adult male**	**Young male**	**Adult female**	**Yearling**	**Lamb**	**Unknown**
Urial	Aug 2013	121	22	344	76	43	111	NA	54	60
Urial	May 2014	13	20	402	50	33	171	26	118	4
Urial	Aug 2014	14	23	302	31	72	93	28	61	17
Urial	Dec 2014	51	21	484	65	36	127	19	116	121
Urial	Mar 2015	48	14	343	29	43	131	3	104	33
Total urial		247	100	1875	251	227	633	76	453	235
Bezoar goat	Aug 2013	121	20	332	26	16	63	NA	55	172
Bezoar goat	Dec 2014	51	19	402	49	40	121	8	105	79
Bezoar goat	Mar 2015	48	6	184	24	22	60	0	74	4
Total bezoar goat		220	45	918	99	78	244	8	234	255

Chapter 6:

A paradox of local abundance amidst regional rarity: the value of montane refugia for Persian leopard conservation

A paradox of local abundance amidst regional rarity: the value of montane refugia for Persian leopard conservation

6.1. Abstract

The population densities of leopards vary widely across their global range, influenced by prey availability, intraguild competition and human persecution. In Asia, particularly the Middle East and the Caucasus, they generally occur at alarmingly low densities. Reliable estimates of population density are important for understanding their ecology and planning their conservation. We used a spatially-explicit photographic capture-recapture (SECR) methodology incorporating animal movement to estimate density for the endangered Persian leopard in three national parks, northeastern Iran. We also investigated the effect of camera trap placement on density estimates. Surprisingly, considering the subspecies' reported low abundance and density based on previous studies, we found relatively high population densities in these three national parks, varying between 4.01 ± SE 1.98 and 8.02 ± SE 2.67 individuals/100 km^2. Estimates for camera-traps placed near water sources were lower and more precise (5.07 ± SE 1.20 leopards/100 km^2) compared with those placed on trails (6.57 ± SE 1.80, leopards/100 km^2), both overlapping the overall estimate (5.90 ± SE 1.10 leopards/100 km^2). The number of adult leopards detected in Tandoureh (30 individuals) was larger than estimated during comparable surveys at any other site in Iran, or indeed globally. We show that attractants such as water and trails can be used to improve the precision of density estimates at lower cost. Finally, our study shows the benefits of protecting even relatively small mountainous areas, which accommodated a high density of leopards and provided refugia in a landscape with substantial human activity.

Key words: spatially-explicit capture recapture, camera trapping, *Panthera pardus saxicolor*, sex-specific movements, water-based sampling.

6.2. Introduction

Resource availability, notably prey density, is the main ‘bottom-up’ process affecting predator density (Karanth et al., 2004; Sharma et al., 2015). Conversely, ‘top-down’ processes, such as disease (Gilbert et al., 2015; Kissui and Packer, 2004), human persecution (Balme et al., 2009b; Williams et al., 2017) and competition (Kortello et al., 2007; Palomares and Caro, 1999) can also operate to shape predator populations. Many apex predators impose top-down regulation on the density of their prey and smaller meso-predators (Ripple et al., 2014; Wallach et al., 2015). Where they compete with larger predators, they are themselves subject to varying degrees of top-down regulation, manifested either in their behavior (Bartnick et al., 2013; du Preez et al., 2015) or population density (but see Balme et al., 2017; Harihar et al., 2011; Linnell and Strand, 2000). The world’s iconic carnivores can be either apex or subordinate predators in different parts of their range. The common leopard *Panthera pardus*, one of the most wide-ranging top predators, frequently illustrates these dual circumstances.

These opposing regulatory processes are likely to have contributed to a great variation in density estimates of leopards across their global range. Estimates of leopard population densities vary 150-fold (see Table 6.S1) from 0 ~ 0.1 individuals per 100 km^2 in northeastern China (Jiang et al., 2015) to 14.9 individuals per 100 km^2 in north central India (Harihar et al., 2009). Leopards may reach particularly high densities in the absence of larger competitors (Grey et al., 2013; Harihar et al., 2011; Mondal et al., 2012). However, while there are numerous studies reporting leopard density, studies from the competitor-free parts of the range in the Middle East and the Caucasus are few (Ghoddousi et al., 2010; Hamidi et al., 2014). Surveys from the protected areas of this region have reported densities at the lowest known extreme for the species, fewer than 0.5 individuals/100 km^2 (Khorozyan et al., 2008; Spalton et al., 2006). Importantly, the few individuals observed in protected areas frequently show no evidence of breeding (Askerov et al., 2015; Avgan et al., 2016; Khorozyan et al., 2014, 2008). Low density and apparent lack of breeding are clearly grounds for conservation concern.

Precise population estimates are important for conservation actions and for monitoring their outcomes (Balme et al., 2009a). The use of camera trap data and capture-recapture methodology is increasingly recognized as a useful method for estimating the density of large carnivores (Royle et al., 2009a). We employed spatially-explicit capture recapture (SECR) methodology (Efford, 2004; Royle et al., 2009b) by means of motion-detector camera traps in three national parks in northeastern Iran to estimate population parameters of the endangered Persian leopard *P.p.saxicolor*.

Maximising the number of captures and recaptures is known to enhance the precision of estimates derived from this methodology. Attractants, such as bait (du Preez et al., 2014) and scent lures can increase capture rates (Braczkowski et al., 2016). However, they may also change animal ranging patterns, potentially violating the ‘closure’ assumption of mark-recapture studies (Gerber et al., 2012), and can also increase the individual, behavioral, and temporal heterogeneity in capture probability (Foster and Harmsen, 2012). Therefore, our second objective was to explore the use of a natural limiting factor as an attractant, i.e. water resources during summer on estimates of population density and composition in leopards. Knowledge of spatial variation in a poorly studied region where populations are likely to be low has implications for leopard conservation in this area.

6.3. Materials and Methods

6.3.1. Study area

The Kopet Dag and Aladagh Mountains in northeastern Iran host a number of montane reserves, including Tandoureh National Park and Protected Area (hereafter NP and PA), Salouk NP & PA and Sarigol NP & PA, lying at the eastern extreme of the Irano-Anatolian Biodiversity Hotspot (E57°15’ to E59°15’, N36° 20’ to N37°20’; Fig 6.1 & Table 6.1). They total 930 km^2 of very rugged mountainous landscapes with steep cliffs and deep valleys (Fig 6.1 & Table 6.1) at altitudes of 1000 to over 3000 meter a.s.l. The climate is temperate semi-arid (Darvishsefat, 2006): mean annual precipitation and temperature are 200 to 300 mm and around $15^{o}C$, respectively.

The vegetation is generally dominated by scrub, particularly *Astragalus* spp. and *Artemisia sieberi*, forming scrub-steppe habitat in most areas, with pockets of juniper *Juniperus* spp. and barberry *Berberis* spp. (Darvishsefat, 2006). Potential ungulate prey for leopards include urial *Ovis orientalis*, bezoar goat *Capra aegagrus*, and wild pig *Sus scrofa*. These areas also support a diverse carnivore community, including leopard, grey wolf *Canis lupus*, striped hyena *Hyaena hyaena*, African wild cat *Felis lybica*, and Pallas's cat *Otocolobus manul* (Ziaie, 2008).

NPs in Iran are subject to stringent law enforcement, and livestock grazing is completely banned. PAs, by contrast have lower levels of protection, and less intense anti-poaching efforts. Furthermore, nomadic pastoralists are permitted to graze their herds in non-NPs during summer (May-August). Their herds are comprised largely of sheep *Ovis aries* (84% ± SE 2) with goats *Capra hircus*. Livestock graze in seasonal pastures in wilderness areas for most of the year, but are herded closer to villages during winter, where they mainly feed on the stubble of crops.

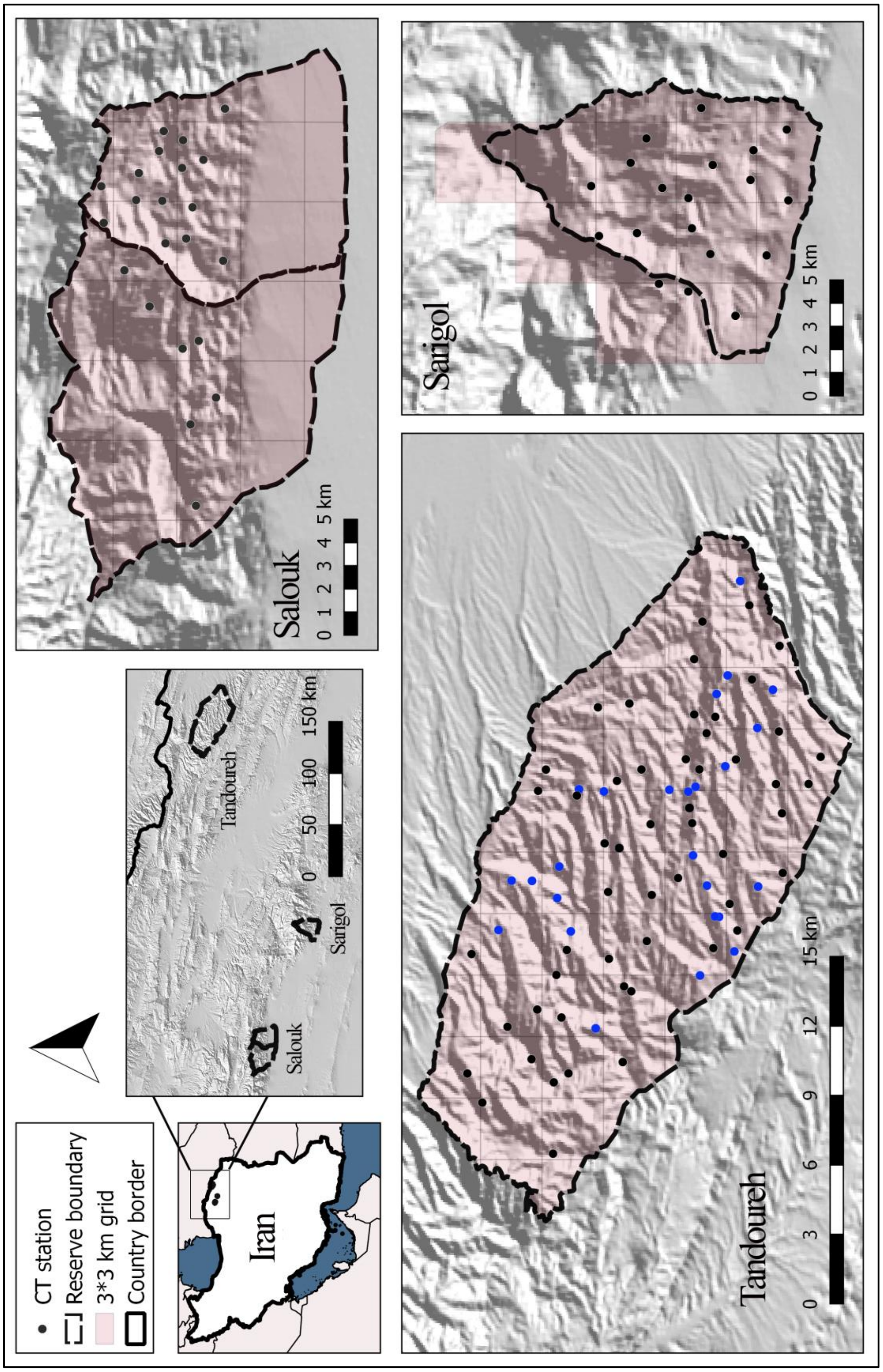

Fig 6.1 Spatial configuration of study areas and locations of camera trap stations across three national parks in northeastern Iran (2015-2016). The map inset shows locations of the study area in Iran. Black circles denote trail-based camera traps while blue circles represent water-based camera traps.

Table 6.1 Details of sampling design for spatially-explicit capture-recapture framework across three study areas in northeastern Iran (2015-2016). MCP = Minimum convex polygon.

Study area	**Area (km^2)**	**Number of stations (#leopard positive stations)**	**Sampling period (days)**	**Effort (trap nights)**	**#available grids (#sampled grids)**	**MCP CT stations (km^2)**	**#stations /grid**
Tandoureh	355.4	80 (50)	31.5 to 25.7.2016 (55)	3597	47 (39)	277.5	2.1 (SE 0.1)
Salouk	199.1	22 (15)	20.10 to 19.12.2017 (60)	1040	17 (11)	50.7	1.8 (SE 0.3)
Sarigol	70.4	19 (17)	22.10 to 16.12.2015 (55)	852	10 (9)	38.4	2.1 (SE 0.3)
Total		119		5410	74 (59)		

6.3.2. Sampling design

We deployed camera traps for 55 to 60 days within each area, a survey effort which is adequate for obtaining reliable estimates (Tobler and Powell, 2013). Each survey period consisted of up to 12 sampling intervals, each lasting for five days. Short intervals enhance the precision of density estimates for rare species such as leopards through avoiding sparse datasets (Goldberg et al., 2015).

We deployed a mean of 2.0 (SE 0.2) camera trap stations on park-wide 3×3 km grids, all with a single camera (Fig 6.1 and Table 6.1). The smallest home range size for a resident male from northeastern Iran is calculated as 63.6 km^2 (minimum convex polygon; Farhadinia et al., 2017). Based on a female/male home range size ratio of 0.4 (du Preez et al., 2014), we assumed a minimum female home range size of approximately 25 km^2. We are therefore confident that all leopards within each study area had at least three camera traps within their range. Camera stations were placed at a mean spacing of 1250 (SE 90), 1400 (SE 87) and 1220 (SE 63) m in Salouk, Sarigol and Tandoureh respectively in order simultaneously to achieve the twin objectives of maximizing the number of individuals caught and adequately recapturing individuals at different camera traps, as required in SECR designs. The dimensions of our trapping grid fall within recommendations for trap spacing relative to movement distributions which stipulate that the separation should be no more than twice the spatial scale parameter to obtain highly precise parameters (Wilton et al., 2014).

In Sarigol and Salouk, camera traps were placed either along ridgelines (n=34, %82.9) or valley bottoms in autumn. In Tandoureh, we used water scarcity in summer to investigate the effect of camera trap placement on detection probability and population size. Accordingly, we established two sampling protocols. All known water resources (springs or artificial waterholes) within each grid cell (n=25, 31.3% of stations) were each equipped with a camera trap. We also placed cameras along trails (n=55, 68.7% of stations), predominantly along ridgelines in Tandoureh. In total, trail-based camera traps predominated (72.3 % of sampling grids) compared with water-based sampling stations (38.3%), because the latter sampling was dictated by the location of water resources. We did not place any camera traps on springs within marginal grids due to the risk of vandalism. Nonetheless, we are confident that approximately half of the national park's known water resources were sampled using camera traps. We did not inform local people of our work, because that could encourage them to look for cameras, and consequently the risk of vandalism would increase.

We deployed Panthera® IV and V (New York, NY 10018, USA) and Cuddeback Capture Model 1125 (Non Typical, Inc., Park Falls, WI, USA), with a minimum delay of 8 seconds between successive photo triggers, all with white flash for high quality night pictures for ease of individual identification. Camera traps were mounted on trees or placed in rock piles, approximately 40 cm off the ground. They were inspected every five to 10 days to ensure their functionality and to check memory cards.

We enquired amongst local conservation officers and rangers for any possible cause of leopard mortality since 2010; only those with hard evidence such as a photo or a carcass were accepted.

6.3.3. Data preparation

The identity of leopards was determined by the unique rosette patterns on their pelage, independently by two researchers (PB and MSF). Sex was distinguished where possible from sex-specific cues, such as visible genitalia or the presence of young (Fig 6.S1). For recaptures, we used an independence threshold of one hour, that is, we discarded all but one capture of the same individual taken at the same camera station within an hour.

6.3.4. Data analysis

We used the package ‘secr’ version 3.0.1 (Efford, 2017) in the *R* environment for statistical computing (R Development Core Team, 2013) to run maximum-likelihood spatially explicit capture-recapture models. The estimates of population density produced by SECR are unbiased by edge effects, incomplete detection and heterogeneous capture probabilities (individual-based, time-based and behaviour-based) and eliminate the need for an ad hoc estimation of the sampling area (Efford, 2004; Royle et al., 2009a).

Density estimates are biased and imprecise if exact detection times are discarded (Borchers et al., 2014). We therefore analyzed our data using a count detector allowing >1 detection per individual per occasion to be entered into the analysis. The programme makes use of three input files: 1) a detection history (information on animal identity, trap location and sampling occasion), 2) trap deployment (location of camera-traps, sampling occasions and camera function details), and 3) potential home-range centres file (a mesh of potential leopard home-range centres located in areas of suitable leopard habitat). The secr package also allowed us to evaluate the effect of covariates (sex and camera placement) on density estimation.

We used a hierarchical model of the temporal emigration phenomenon, composed of an explicit state-space process model and an observation model (Royle and Young, 2008). The animal population size and their respective central locations (“home-range centers”) constitute the state-space process, assuming a Poisson distribution (Royle et al., 2009b).

The observation model describes the probability of encounter as a function of an individual's location at the time of sample, and a probability of detector parameter (Royle et al., 2009b). Capture probability was estimated using an exponential detection model as a function of distance from the activity centres. The detection function contains two parameters: g0, (the intercept) (i.e. the baseline detection probability when the distance between the animal's activity center and the camera traps is zero), and σ which is the spatial scale parameter (m) of the encounter probability model and is related to animal movement (Royle et al., 2009b; Royle and Young, 2008).

The state-space model was described by equally spaced points in a regular grid, with a mesh size of 1 km^2. A buffer was plotted around the trap array to incorporate individuals with activity centers outside of the trapping area, but whose movement range extends into the sampling area (Efford, 2004; Royle and Young, 2008). We then applied a 40 km buffer around a homogenous distribution of potential home-range centres and multiple models were run for buffers with smaller sizes to detect the point at which the density estimate stabilised. Non-habitat areas such as villages, farmlands, and open areas were masked out from the effective area, based on GPS relocation data of collared leopards (Farhadinia et al., 2017) and our field knowledge. Home range centres falling over non-suitable habitats were mapped using Quantum GIS (QGIS Development Team, 2017) and Google Earth Pro 7.1.7.2606 (Google Inc., USA) and were marked accordingly in the secr input file.

The buffer in each area, Tandoureh=12 km, Sarigol= 7 km and Salouk=14 km, appeared to be adequate as further increases did not affect density estimates. The size of the buffer was almost equal to 5σ in each area which corresponds to an area that would contain 99.99% of captured animals (Borchers and Efford, 2008). This resulted in an effective sampling area of 508.6, 124.8 and 299.3 km^2, respectively (Table 6.1).

Our survey period (≤ 2 months) seemed sufficiently short to assume demographic closure, based on previous studies of leopards (Balme et al., 2009a; Braczkowski et al., 2016). We assessed geographic closure using the “closure.test” function in secr package, based on the assumption of heterogeneity in recapture probability (Otis et al., 1978).

We fitted a suite of eight models to the data for each area to assess the impact of potential trap-specific responses of individuals and sex-specific detection probability for density estimates (full secr model specifications in Table 6.2). For the Tandoureh site, we included placement of camera traps (water versus trail) as a habitat covariate and three additional models were fitted (Table 6.2).

The models were fitted by maximising the likelihood conditional on the number of animals detected. An Information Theoretic (IT) approach (Burnham and Anderson, 2002) was used to rank candidate models in secr using Akaike’s Information Criterion (AIC). The highest ranked models (all within 2.0 ΔAIC of the top model, averaged if multiple models were selected) were used to estimate leopard density, capture probability at home-range centre (g0), and sigma.

We also used a Negative Binomial regression to explore the variation in detection frequency across the three areas implemented in the ‘MASS’ package (Venables and Ripley, 2002). We tested for goodness-of-fit for the regression model with a chi-square test based on the residual deviance and degrees of freedom. A goodness-of-fit *P* value greater than 0.05 was considered as indicative of an acceptable fit.

Table 6.2 Model definitions used in secr to generate density estimates of leopards across multiple study areas in northeastern Iran (2015-2016).

Model Number	Variable	Variable function	Effect
1	g0~1	g0 and sigma kept constant	g0 is constant across animals, occasions and detectors
2	g0~b	Step change in parameter after initial detection of animal	Learned response affects g0
3	g0~h2	Finite mixture model with two latent classes	2-class finite mixture for heterogeneity in g0
4	g0~t	One level for each occasion	g0 vary due to time
5	g0~bk	Site-specific step change	Animal x site response
6	g0~Bk	Time-specific transient response	Animal x site response
7	g0~Sex	Step change with animal sex	Male and females differ in g0
8	sigma~Sex	Sex specific detection	Detection scale sigma differs between sexes, while g0 is constant
9	g0~Placement	Step change with camera trap placement type	Detector placement affects g0
10	sigma~Placement	Camera trap placement-dependent detection scale	Detection scale sigma differs based on detector placement
11	g0~Placement, sigma~Placement	Camera trap placement-specific step change and detection scale	Both detection parameters are affected by detector placement

In order to test the effect of sampling on density estimate precision, we then fitted eight secr models for detection histories of each sampling method (25 water-based and 55 trail-based stations) to compare their specific density and detection parameters. In order to illuminate the effect of sampling and age/sex group on detection frequency in leopards in Tandoureh, we then fitted generalized linear mixed models (GLMM) with a Poisson error distribution using the 'lme4' package (Bates et al., 2016). We used the default Laplace Approximation for estimating maximum likelihood to fit two interactive and additive models between age/sex groups and sampling type. Leopard identity was included as a random effect. We checked the models for over-dispersion (i.e. the ratio of residual deviance to degrees of freedom). Also, normal distribution of residuals and homoscedasticity were checked for fitted models. The significance of terms in the final model was assessed using log-likelihood ratio tests for comparing the goodness of fit between models. We then used least-squares means to predict the effects of sampling type on each different age/sex group from the final GLMM model using 'lsmeans' package (Lenth, 2016). Finally, we used Fisher's exact test to test the null hypothesis that the proportion of different age and sex classes detected are independent of whether a camera is placed on water or trail in Tandoureh.

6.4. Results

We obtained a total of 1335 pictures containing 477 leopard detections during 5410 trap nights across the three study areas (Table 6.1). The number of adult leopards identified using the right flank was more than that identified from the left flanks at three sites, resulting in an overall number 52 vs. 44 individuals (23 males, 20 females and 9 with unknown sex based on right flank identified individuals, Table 6.3).

The median number of captures per individual was 1.5 (range: 1–18 captures), 2.0 (range: 1-15), and 3.0 (range: 1-33) in Sarigol, Salouk and Tandoureh, respectively. The goodness-of-fit test for the Negative Binomial regression for comparing detection frequencies across three study areas had a good fit (residual deviance = 53.67, df= 49, *P*=0.29). This model indicated that leopards in Tandoureh had a higher detection frequency compared with the other two sites (Z=2.33, *P*=0.02). Five, three and 12 individuals were captured only once in Sarigol, Salouk and Tandoureh, respectively, equal to 25 to 50% of total adult numbers.

We also detected seven leopard families with a total of 10 cubs (Table 6.3), equal to 9.1% to 19.4% of total number of detected individuals in each area. In Tandoureh where we had multiple detections of each leopard family (n=35 for five families), cubs were photographed with the female only in 19 cases (54.3%) whereas the adult was the only representative of each family in the rest of the detections (n=16, 45.7%). The closure test suggested no violation of the population closure assumption (Tandoureh: Z=-0.73, *P*=0.23; Salouk: Z=-1.40, *P*=0.08; Sarigol: Z=0.24, *P*=0.60).

Table 6.3 Details of baseline information on leopards based on systematic camera trapping across three study areas in northeastern Iran (2015-2016).

Study area	**#leopard pictures**	**#independent leopard detections (#non-identifiable)**	**#detected adult individuals**		**Sex ratio (n)**	**#families**	**#dependent cubs**
			Right flank	**Left flank**			
Tandoureh	1097	354 (67)	30	26	1.1:1 (29)	5	7
Salouk	99	56 (9)	12	10	1:1 (8)	1	2
Sarigol	139	67 (18)	10	8	1:0.5 (6)	1	1
Total	1335	477	52	44		7	10

A total of 11 confirmed leopard mortalities were obtained for 2010-2016, mostly in Tandoureh (n=9) as well as one for each other two study areas. Two leopards were allegedly killed in retaliation for livestock depredation by communities, while the majority of leopards' causes of death were unknown.

6.4.1. Density estimates

From the models we created for each survey in secr, there was very strong support for only one model based on the AIC. Model comparison showed that the site-specific behavioural response in the baseline detection probability model (g0 ~bk) fitted the Tandoureh survey data best while the site-specific transient response model (g0 ~Bk) performed better for Sarigol. Both site-specific response models (bk and Bk) are similar, but the latter tests the hypothesis that leopard behaviour changes after being detected at a specific site, but only for that site and not for the duration of the survey (Zimmermann and Rovero, 2016). The M_{bk} in Tandoureh indicated that when a leopard is detected in a specific camera trap site, the probability of a subsequent encounter for the entire survey is increased, i.e. the individual becomes ‘trap happy’ (g0.bkTRUE=2.2, CI95% 1.6-2.8). In contrast, g0 (BkTRUE) was -3.0 (CI 95% -6.1-0.1) in Sarigol and its marginal overlapping with zero suggested a general tendency to become ‘trap shy’ at the population level. In contrast, the temporal variation in trappability model (g0~t) ranked first in AIC weight in Salouk, implying that detection probability varied among occasions.

The best performing model (Table 6.4), produced a density estimate of 5.90±1.1 leopards/100 km^2 for Tandoureh (g0=0.19±0.03; σ = 1704±141 m). The secr density (SE) estimate for Salouk survey was 4.0±2.0 leopards/100 km^2 (g0=0.30±0.13; σ=2774±1018 m). The density estimates were highest in Sarigol, i.e. 8.02±2.67 leopards/100 km^2 with detection function parameters of g0=0.77±0.22 and σ=994±151 m (Table 6.5).

Sex-specific density estimates were similar in Salouk and Tandoureh, whereas the density of males was two-fold that of females’ in Sarigol (Table 6.5). All pairwise σ_{Male} and σ_{Female} comparisons showed overlapping confidence intervals (Table 6.5).

Table 6.4 Characteristics of candidate models generated in secr for Persian leopards in three study areas in northeastern Iran (2015-2016) g0=detection probability at trap location considered as home range center, sigm=spatial scale parameter, pmix=mixing parameter and EX=exponential detection function.

Model description	Detection function	Parameters	Log likelihood	AIC	ΔAIC	AIC weight
Tandoureh						
g0~bk, sigma~1	EX	3	-903.8	1813.5	0.0	1
g0~CTPlacement, sigma~CTPlacement	EX	4	-908.6	1825.2	11.6	0
g0~CTPlacement, sigma~1	EX	3	-910.8	1827.5	14.0	0
g0~Bk, sigma~1	EX	3	-924.1	1854.1	40.6	0
g0~1, sigma~CTPlacement	EX	3	-928.4	1862.7	49.2	0
g0~h2, sigma~1 pmix~h2	EX	4	-927.8	1863.7	50.1	0
g0~t, sigma~1	EX	12	-927.9	1879.8	66.3	0
g0~b, sigma~1	EX	3	-940.5	1886.9	73.4	0
g0~1, sigma~1	EX	2	-942.5	1889.0	75.5	0
g0~Sex, sigma~1	EX	3	-942.1	1890.3	76.7	0
g0~1, sigma~Sex	EX	3	-942.2	1890.5	76.9	0
Salouk						
g0~t, sigma~1	EX	13	-171.5	369.1	0.0	0.98
g0~1, sigma~Sex	EX	3	-186.0	378.0	8.9	0.01
g0~1, sigma~1	EX	2	-187.1	378.2	9.1	0.01

g0~bk, sigma~1	EX	3	-186.9	379.8	10.7	0.00
g0~Bk, sigma~1	EX	3	-187.0	380.0	10.9	0.00
g0~Sex, sigma~1	EX	3	-187.1	380.2	11.1	0.00
D~1, g0~b, sigma~1	EX	4	-186.3	380.6	11.5	0.00
g0~h2, sigma~1, pmix~h2	EX	4	-186.3	380.6	11.5	0.00
Sarigol						
g0~Bk, sigma~1	EX	3	-150.5	307.0	0.0	0.52
g0~t, sigma~1	EX	12	-142.8	309.5	2.5	0.15
g0~bk, sigma~1	EX	3	-151.8	309.7	2.7	0.14
g0~Sex, sigma~1	EX	3	-152.3	310.6	3.6	0.09
g0~1, sigma~1	EX	2	-153.4	310.7	3.7	0.01
D~1, g0~b	EX	4	-153.3	314.6	7.6	0.01
g0~h2, sigma~1, pmix~h2	EX	4	-153.4	314.7	7.7	0.01
g0~1, sigma~Sex	EX	3	-157.7	321.5	14.5	0.00

Table 6.5 Density estimates of Persian leopards with standard error (SE) and 95% confidence interval (lower and upper) of parameters for spatially-explicit capture recapture models fit to camera trapping data from three study areas in northeastern Iran (2015-2016). Density is reported in independent leopards per 100 km^2. The g0 is the detection probability at trap location considered as home range center and σ is the scale of an individual's movement distribution in meters.

Parameter	**Estimate**	**SE**	**Lower CI**	**Upper CI**
Sarigol				
g0	0.77	0.22	0.22	0.98
σ_{Overall}	994	151	739	1336
σ_{Male}	1242	280	804	1920
σ_{Female}	909	108	720	1146
$\text{Density}_{\text{Overall}}$	8.02	2.67	4.24	15.15
$\text{Density}_{\text{Male}}$	3.21	1.64	1.25	8.24
$\text{Density}_{\text{Female}}$	1.60	1.15	0.45	5.66
Salouk				
g0	0.30	0.13	0.11	0.58
σ_{Overall}	2774	1018	1382	5566
σ_{Male}	3588	1296	1806	7126
σ_{Female}	1272	460	639	2530
$\text{Density}_{\text{Overall}}$	4.01	1.98	1.60	10.02
$\text{Density}_{\text{Male}}$	1.34	0.86	0.42	4.22
$\text{Density}_{\text{Female}}$	1.34	0.86	0.42	4.22

Tandoureh				
g0	0.19	0.03	0.13	0.26
$\sigma_{Overall}$	1704	141	1449	2004
σ_{Male}	1748	207	1386	2206
σ_{Female}	1592	201	1245	2036
Density $_{Overall}$	5.90	1.10	4.09	8.49
Density $_{Male}$	2.95	0.77	1.78	4.89
Density $_{Female}$	2.75	0.75	1.63	4.63

6.4.2. Effects of sampling design

Water-based sampling increased detections per individual by 2.3-fold in Tandoureh (water-based=7.9±1.5 versus trail-based=3.4±0.7, Fig 6.2), but the number of adult individuals detected at water was slightly lower (20 on-water versus 24 on-trails). In total, 9 adult leopards were detected only once on cameras placed at trails whereas it was only four individuals at water resources.

There was strong support for a site-specific behavioural response in the baseline detection probability model (g0~bk) for water-based sampling based on the AIC (Table 6.S2). In contrast, three models performed better when trail-based camera trapping models were compared. Sex was an important covariate to determine both detection parameters, particularly the spatial scale parameter (Table 6.S2), resulting in different sex-specific sigma (σ_{Male}=1830±260 m versus σ_{Female}=1022±431 m, Table 6.6).

Table 6.6 Detection frequencies, density estimates and detection parameters (g0 and σ) with standard error compared between trail and water-based sampling in Tandoureh National Park. The g0 is the detection probability at trap location considered as home range center and σ is the scale of an individual's movement distribution in meter.

	Camera trap placement		
Parameter	**Water**	**Trail**	**Water/trail ratio**
Detection frequency (SE)/individual*			
Adult male	8.1 (2.4)	4.8 (1.1)	1.7
Adult female	8.9 (2.7)	1.9 (0.5)	4.7
Cub	5.8 (2.8)	1.0 (0.0)	5.8
Density±SE (leopards/100 km^2)	5.07±1.20	6.57±1.80	0.8
$g0_{\text{Overall}}$	0.30±0.07	0.18±0.07	1.7
$g0_{\text{Male}}$	0.38±0.14	0.18±0.07	2.1
$g0_{\text{Female}}$	0.26±0.08	0.18±0.07	1.4
σ_{Overall}	1749±200	1014±422	1.7
σ_{Male}	1577±290	1830±260	0.9
σ_{Female}	1836±270	1022±431	1.8

* A single individual with unknown sex was excluded from sampling comparison.

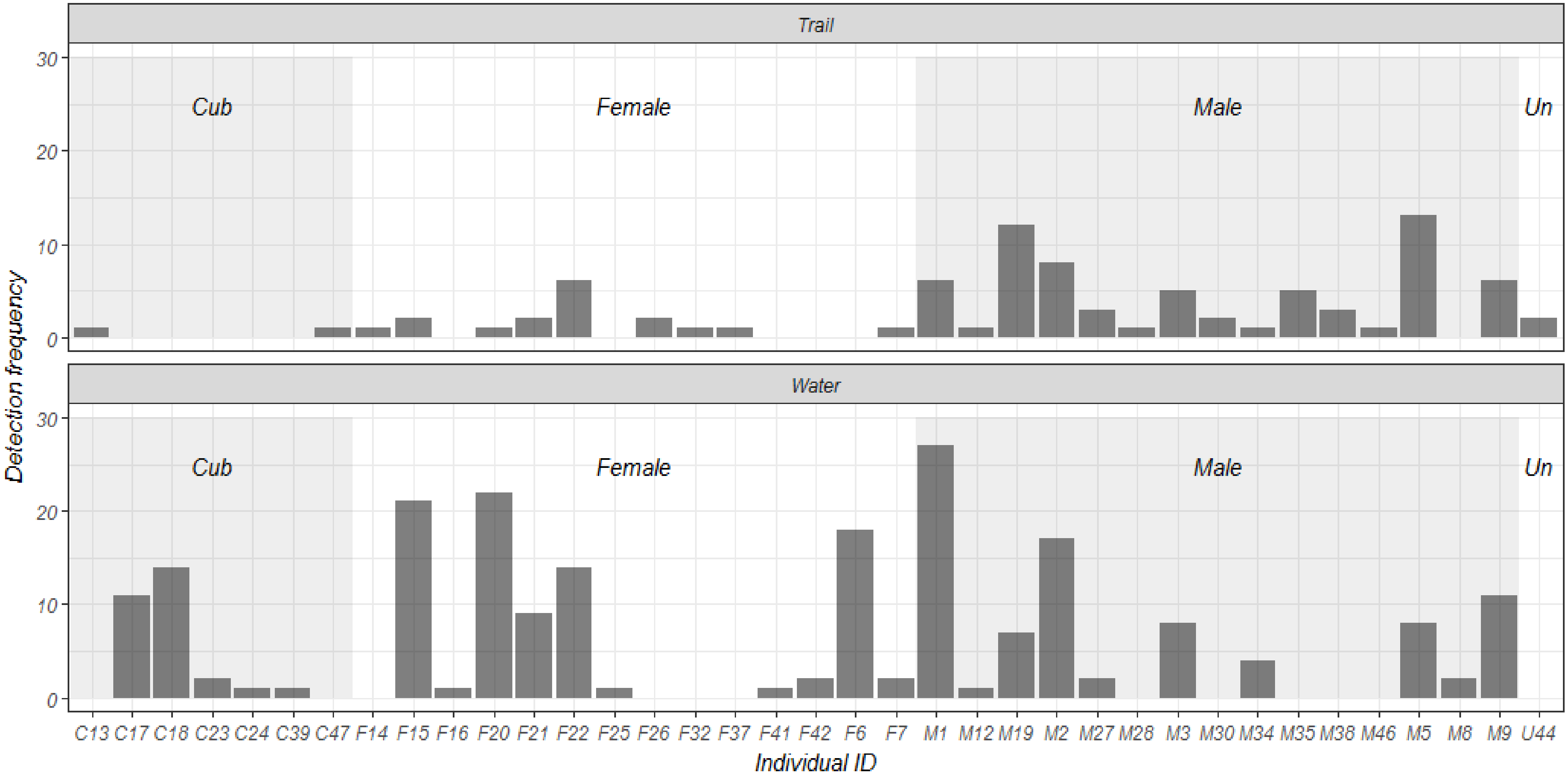

Fig 6.2 Comparison of detection frequency for all demographic classes between water and trail-based camera traps. Each code on the x-axis refers to a single individual leopard within the relevant demographic class; M=male, F=female, U and Un=unidentifiable and C=cub.

Water-based sampling produced lower density estimates with higher precision (5.07±1.20 leopards/100 km^2) compared with trail-based camera trapping (6.57±1.80 leopards/100 km^2, Table 6.6). Both sampling regimes' standard errors overlapped with the overall density estimate (5.90±1.10 leopards/100 km^2). Detection probability was 1.7 times higher for water-based sampling compared with trail-based camera trapping (g0=0.30 vs. 0.18). None of the sampling methods showed any sex-specific difference in detection probability (g0) (Table 6.6).

There was no evidence that the total number of unique individuals detected from each age/sex group varied with sampling method (Fisher's exact test, $P=0.40$). The frequency of detection per individual, however, was higher for each age/sex group for water-based cameras (Fisher's exact test, $P<0.001$; Fig 6.3). There was evidence that the effect of camera-trap placement on detection success varied among age/sex groups (interaction term $X^2=16.82$, df=3, $P<0.001$). While all age/sex classes were more frequently detected at water-based cameras, this ranged from 1.7 times more in adult males ($Z= -2.31$, $P=0.29$) to 4.7 times more in adult females ($Z= -5.69$, $P<0.001$, Fig 6.3 and Table 6.6). Cubs were mostly detected at water resources, both in terms of numbers and the detection per each individual cub; however, the placement effect did not differ between water versus trail-based sampling ($Z= -1.43$, $P=0.84$).

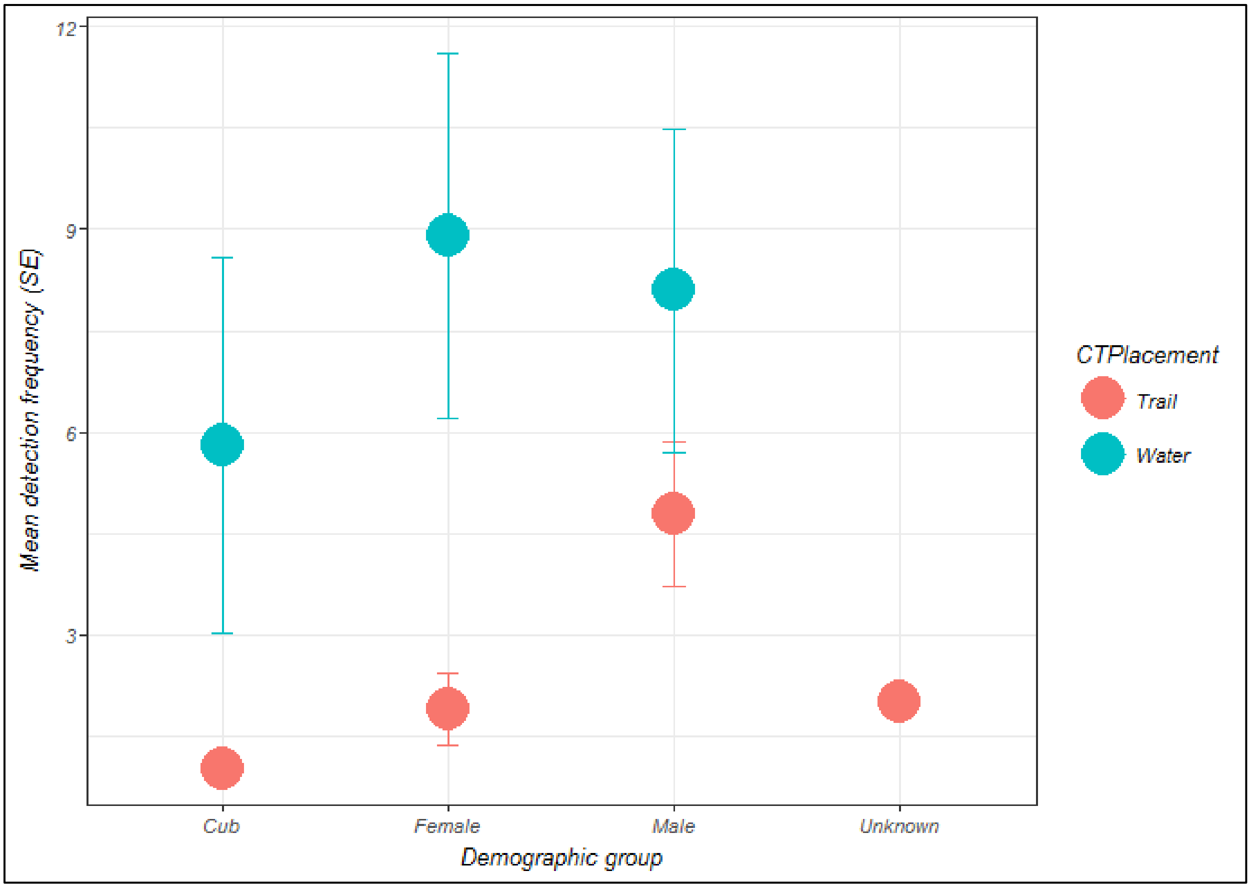

Fig 6.3 Mean detection frequency per individual of demographic classes between water and trail-based camera traps.

6.5. Discussion

We documented the highest densities of leopards in the Middle East and the Caucasus, as well as the largest number of leopards detected at any location surveyed to date. The water-based sampling method yielded lower density estimates but with higher precision than trail-based method. Our findings highlight the importance of northeastern Iran as a leopard hotspot, and thus a focus of conservation.

6.5.1. Ecological implications

Leopard density estimates in northeastern Iran far exceeded estimates made elsewhere in the Middle East and the Caucasus (Hamidi et al., 2014; 0.34 to 2.63 individuals/100 km2; Khorozyan et al., 2008). Our estimates were also higher than the majority of published leopard densities across the species' continental Asian range (Fig 6.4 and Table 6.S1), except a few protected sites in India (Goldberg et al., 2015; Kalle et al., 2011).

There are three possible hypotheses explaining the high density of leopards within our study areas. First, there is a negative relationship between human encroachment around protected areas and leopard population density (Balme et al., 2010; Rosenblatt et al., 2016). Although we do not have density estimates from outside the NPs, the high wild prey abundance inside the NPs compared with non-NPs (based on ranger inquiries) support this hypothesis. Therefore, the high number of leopards suggests that our sites are robust refugia for leopards.

Alternatively, population perturbation due to excessive persecution can result in many transient individuals (Balme et al., 2009b). Our confirmed mortality records do not imply excessive persecution; however, illegal behaviour such as persecuting a protected species often occurs elusively. Therefore, our mortality records perhaps do not properly reflect the extent of persecution.

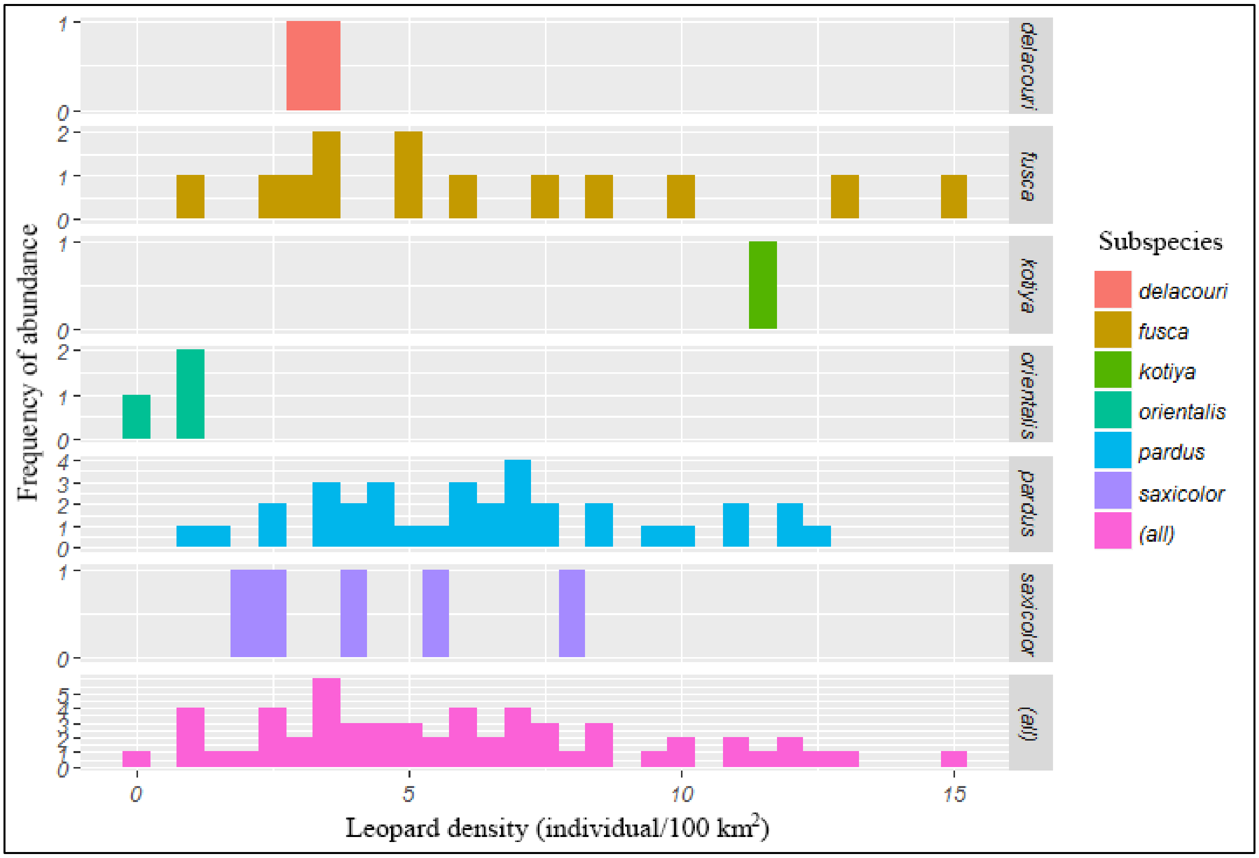

Fig 6.4 Distribution of density estimates for leopard subspecies across the species global range.

Finally, our study areas possibly represent a relatively stable resident population with a higher than usual proportion of transients looking for territory. In the absence of a minimal wild prey base outside the NPs, transients may move into the NPs. This is consistent with the high home range overlap between conspecifics observed in Tandoureh (chapter 4). We were unable to accurately age individuals using camera trap photos (because of the variation in camera angle, light level and leopard orientation). We cannot therefore evaluate the proportion of young individuals, the age group most likely to disperse (Fattebert et al., 2015).

While our data tend to support the first hypothesis, we have insufficient evidence to reject the other two. To rigorously test the second and third hypotheses, it would necessitate a multiple-year camera trapping assessment of population stability (hypotheses 2 and 3) and a longitudinal tracking study documenting the occurrence of young transients (hypothesis 3).

6.5.2. Methodological applications

The relative precision of σ_{Male} and σ_{Female} indicates that our sampling may have provided a sufficient number of spatial recaptures to estimate this parameter for both sexes (Goldberg et al., 2015). We found no evidence that detection probability (g0) varied between sexes. This contradicts with previous SECR estimates on leopards (Braczkowski et al., 2016; Goldberg et al., 2015; Gray and Prum, 2012) where males were more likely to be detected. Sexual patterns of space-use among large cats are widely considered to differentiate SECR parameters and so influence density estimates (Gray and Prum, 2012; Sollmann et al., 2011; Tobler and Powell, 2013). Differences in habitat accessibility may explain this, as it is less likely that leopards use steep cliffs for moving in the landscape in rugged terrain, using instead ridgelines and valley bottoms, resulting in no evident sex-specific differences in detection probability. Similarly, the detection probability did not vary between the sexes in another montane big cat, the snow leopard *P. uncia* (Sharma et al., 2014). Forest or lowland landscapes, by contrast, may allow large cats to partition space use differently between the sexes, resulting in different detectabilities (Braczkowski et al., 2016; Gray and Prum, 2012; Wearn et al., 2013).

Dependent cubs have generally low capture probabilities in leopard population studies. Similarly, behavioural differences between cubs and adults such as deliberate avoidance of camera traps can result in cubs having extremely low capture probabilities in tigers *P. tigris* (Karanth and Nichols, 1998). Attractant-based camera trapping, such as the use of water resources in this study or baits (du Preez et al., 2014), can substantially improve the detection of cubs. Although the proportion of cubs in the population in northeastern Iran was higher than almost all available estimates from different subspecies of leopards (Table 6.S1), mother leopards in almost half of detections were photographed without their cubs. Lower mobility of younger leopard cubs (Balme et al., 2013) and the female habit of hunting alone (Wilmers et al., 2017) may explain the lower detectability of cubs.

The two sampling methods yielded different density estimates and precision: we achieved marginally lower density and moderately higher precision using water-based sampling. There are likely to be two underlying reasons. First, the higher number of trail stations compared to water-based cameras (55 vs. 25) and the spatial configuration of trail cameras which provided more coverage (72.3% vs 38.2% of sampling grids) resulted in more individuals detected along the trails than at water resources (24 vs. 20). Second, cameras at water resources achieved a higher recapture rate (7.9±1.5 vs. 3.4±0.7 captures per individual) and a lower number of individuals with single detections (4 vs. 9 individuals).

The use of attractants such as scent lures or baits involves some extra cost. Arguably, this has a negligible effect on population density estimates (Braczkowski et al., 2016), but may slightly increase precision estimates for leopard populations (du Preez et al., 2014). Our water-based sampling suggested that a considerable improvement in the precision of density estimates can be achieved with significantly lower operational costs. We recommend a mixed strategy prioritizing water resources over trails wherever possible (where for example the risk of theft or vandalism is low), while covering the remaining areas using trail-based camera stations. As a consequence of the concentration of cameras and leopards around water sources in hot months resulted in a positive trap response in Tandoureh, which is commonly seen in baited sampling surveys (du Preez et al., 2014; Royle et al., 2011).

We acknowledge two possible sources of bias in our density estimates. First, when the camera spacing is larger than the smallest female home range radius, density estimates are negatively biased (Tobler and Powell, 2013). It is unlikely that our assumption of 25 km^2 for minimum female home range is an overestimation compared to global patterns in home range size of leopards (Marker and Dickman, 2005). The camera trap spacing therefore is less likely to cause any bias in our estimates. Second, a trapping array smaller than the average home range can positively bias the density estimate (Maffei and Noss, 2008; Tobler and Powell, 2013) due to temporary emigration (Royle and Young, 2008). High chance of vandalism outside the national parks' boundaries delimited our trap array, except in Salouk. As such, density estimates in Salouk and Sarigol tend to suffer a positive bias of approximately 30%, based on the home range/trap array size ratio (Tobler and Powell, 2013). The high detection probability at home range centers and the small movement parameter in Sarigol suggest that the trap array has perhaps captured the edges of many home ranges (Maffei and Noss, 2008). In contrast, our estimate for Tandoureh is less likely to suffer from any of the two biases.

6.5.3. Conservation implications

Eight Asian subspecies of leopards have experienced between 83 and 87% range loss (Jacobson et al., 2016), and often now occur at perilously low densities (see Table 6.S1). Conservation interventions to halt, and reverse, these declines, and monitor recovery, require unbiased and precise estimates of population density. Our study provides a protocol for achieving this, while mindful of minimizing operational costs. This is particular timely in the Middle East and the Caucasus, where several of the last remnant populations of leopards occur along international borders, such as Iraq, Turkey and Iran (Avgan et al., 2016), the Lesser Caucasus (Askerov et al., 2015; Farhadinia et al., 2015), the Kopet Dag Mountains along the Iran-Turkmenistan border

(Atamuradov et al., 1999; Farhadinia et al., 2017), and Yemen, Oman and Saudi Arabia (Spalton et al., 2006). Unfortunately, many of these areas are badly affected by military conflicts and security concerns (Hanson et al., 2009), which are not currently attractive for conservation investment.

The high population density of leopards observed in this study, contrary to expectation, illustrate that an area as small as the home range of a single individual (Farhadinia et al., 2017) can provide sanctuary for a high density of leopards. Importantly, controlling two regulatory processes, prey depletion and leopard poaching are crucial for such an achievement.

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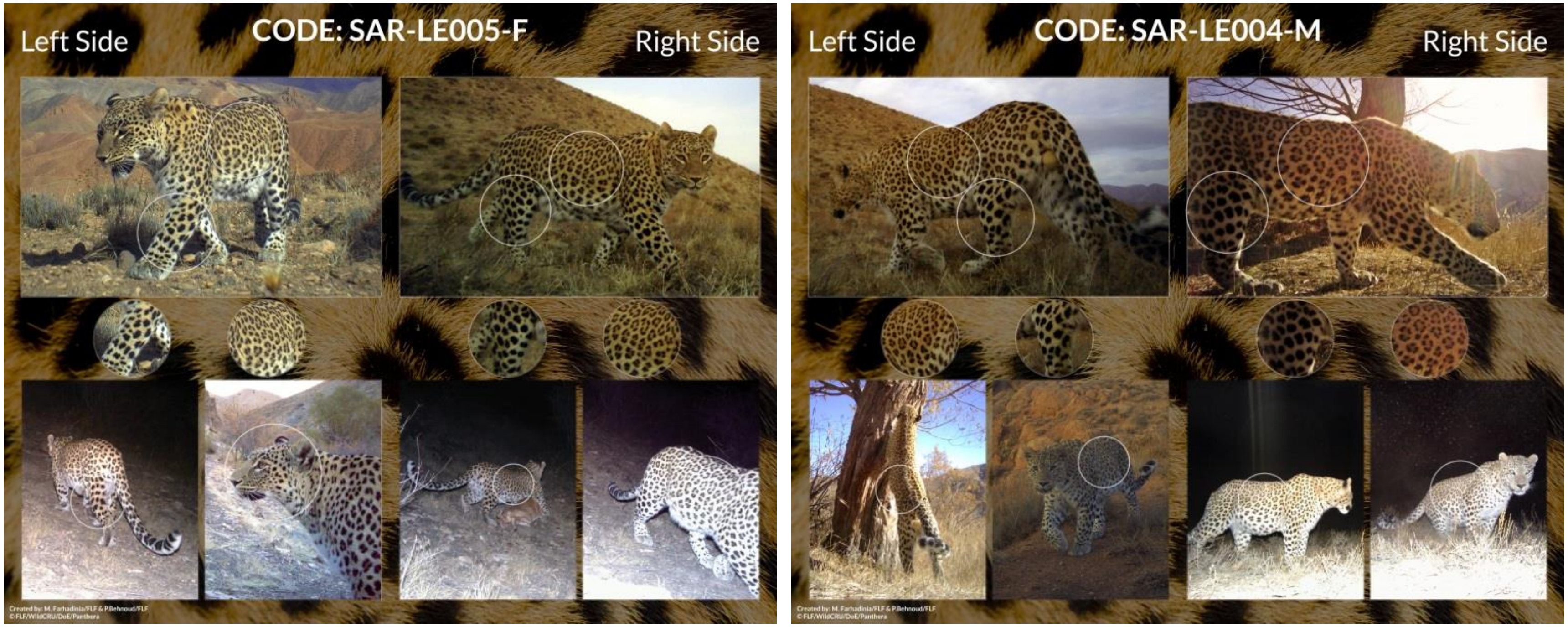

Fig 6.S1 Identification of leopard individuals based on the photos from their right or left flanks. Here, four individuals are shown.

Table 6.S1 A review of published density estimates using photographic capture-recapture methodology on leopard across its global range.

Number	Country	Continent	Subspecies	Study area	Density (individual/100 km^2)	#adults	#cubs	Cub/Total population	Study
1	Cambodia	Asia	*delacouri*	Mondulkiri Protected Forest	3.6	12	0	0	(Gray and Prum, 2012)
2	Malaysia	Asia	*delacouri*	Taman Negara	3	9	NA	NA	(Hedges et al., 2014)
3	India	Asia	*fusca*	Cilla forest	14.9	8	Yes	NA	(Harihar et al., 2009)
4	Bhutan	Asia	*fusca*	Jigme Singye Wangchuck National Park	1.04	25	NA	NA	(Wang and MacDonald, 2009)
5	India	Asia	*fusca*	Pakke Tiger Reserve, Arunachal Pradesh	2.82	7	NA	NA	(Selvan et al., 2014)
6	India	Asia	*fusca*	Akole	4.8	11	NA	NA	(Athreya et al., 2013)
7	India	Asia	*fusca*	Sariska (2007)	8.4	19	NA	NA	(Mondal et al., 2012)
8	India	Asia	*fusca*	Sariska (2008)	7.4	17	NA	NA	(Mondal et al., 2012)
9	India	Asia	*fusca*	Sariska (2009)	5.2	14	NA	NA	(Mondal et al., 2012)
10	India	Asia	*fusca*	Sariska (2010)	2.3	10	NA	NA	(Mondal et al., 2012)
11	India	Asia	*fusca*	Sariska (2011)	5.8	14	NA	NA	(Mondal et al., 2012)
12	Nepal	Asia	*fusca*	Bhabhar of Terai Arc	3.7	19	0	0	(Thapa et al., 2014)
13	India	Asia	*fusca*	Mudumalai Tiger Reserve	13.1	29	Yes	NA	(Kalle et al., 2011)

14	Bhutan	Asia	*fusca*	Royal Manas National Park	10	22	NA	NA	(Goldberg et al., 2015)
15	India	Asia	*fusca*	Manas	3.4	27	NA	NA	(Borah et al., 2014)
16	India	Asia	*fusca*	Churna (1)	8	10	2	0.17	(Edgaonkar, 2008)
17	India	Asia	*fusca*	Churna (2)	9.3	11	2	0.15	(Edgaonkar, 2008)
18	India	Asia	*fusca*	Kamti	7.5	11	0	0	(Edgaonkar, 2008)
19	India	Asia	*fusca*	Lagda	7.3	8	0	0	(Edgaonkar, 2008)
20	Sri Lanka	Asia	*kotiya*	Horton Plains National Park	11.7	8	1	0.11	(Kittle et al., 2017)
21	Sri Lanka	Asia	*kotiya*	Ruhuna (Yala)	12.3	15	NA	NA	(Kittle et al., 2017)
22	Sri Lanka	Asia	*kotiya*	Ruhuna (Yala)	14.7	19	NA	NA	(Kittle et al., 2017)
23	Sri Lanka	Asia	*kotiya*	Ruhuna (Yala)	10	14	NA	NA	(Kittle et al., 2017)
24	Sri Lanka	Asia	*kotiya*	Ruhuna (Yala)	11.4	20	NA	NA	(Kittle et al., 2017)
25	Russia	Asia	*orientalis*	Primorski Krai (N unit)	1.1	9	0	0	(Kostyria et al., 2003)
26	Russia	Asia	*orientalis*	Primorski Krai (S unit)	1.03	8	0	0	(Kostyria et al., 2003)
27	China	Asia	*orientalis*	Northeastern China	0.05	10	1	0.09	(Jiang et al., 2015)
28	South Africa	Africa	*pardus*	Mkhuze (2005)	11.11	13	NA	NA	(Balme et al., 2010)
29	South Africa	Africa	*pardus*	Mkhuze (2008)	10.76	12	NA	NA	(Balme et al., 2010)

30	South Africa	Africa	*pardus*	Non-PAs (2005)	2.49	4	NA	NA	(Balme et al., 2010)
31	South Africa	Africa	*pardus*	Phinda (2005)	7.17	13	NA	NA	(Balme et al., 2010)
32	South Africa	Africa	*pardus*	Phinda	6.97	13	1	0.07	(Balme et al., 2009a)
33	South Africa	Africa	*pardus*	Phinda (2005)	7.17	13	1	0.07	(Balme et al., 2009b)
34	South Africa	Africa	*pardus*	Phinda (2007)	9.42	13	1	0.07	(Balme et al., 2009b)
35	South Africa	Africa	*pardus*	Phinda (2009)	11.21	13	1	0.07	(Balme et al., 2009b)
36	Namibia	Africa	*pardus*	Waterberg Plateau Park	1	4	NA	NA	(Stein et al., 2011)
37	Namibia	Africa	*pardus*	Waterberg Plateau Farm	3.6	10	NA	NA	(Stein et al., 2011)
38	Gabon	Africa	*pardus*	Central Gabon (2)	2.69	4	NA	NA	(Henschel, 2008)
39	Gabon	Africa	*pardus*	Central Gabon (3)	4.58	8	NA	NA	(Henschel, 2008)
40	Gabon	Africa	*pardus*	Central Gabon (4)	12.08	15	NA	NA	(Henschel, 2008)
41	South Africa	Africa	*pardus*	Lapalala, Waterberg Biosphere	5.35	12	NA	NA	(Swanepoel et al., 2015)
42	South Africa	Africa	*pardus*	Farming matrix, Waterberg Biosphere	6.59	12	NA	NA	(Swanepoel et al., 2015)
43	South Africa	Africa	*pardus*	Welgevonden, Waterberg Biosphere	4.56	18	NA	NA	(Swanepoel et al., 2015)
44	South Africa	Africa	*pardus*	Zululand Rhino Reserve	7	6	2	0.25	(Chapman and Balme, 2010)
45	South Africa	Africa	*pardus*	N’wanetsi concession, Kruger	12.7	14	NA	NA	(Maputla et al., 2013)
46	South Africa	Africa	*pardus*	Western Shore	8.42	23	0	0	(Ramesh et al., 2017)
47	South Africa	Africa	*pardus*	Eastern Shore	7.4	16	0	0	(Ramesh et al., 2017)

48	South Africa	Africa	*pardus*	Tembe	4.8	25	0	0	(Ramesh et al., 2017)
49	South Africa	Africa	*pardus*	Ndumo	1.6	3	2	0.4	(Ramesh et al., 2017)
50	South Africa	Africa	*pardus*	Phinda	3.4	15	2	0.12	(Braczkowski et al., 2016)
51	South Africa	Africa	*pardus*	Phinda	3.47	14	2	0.13	(Braczkowski et al., 2016)
52	Zimbabwe	Africa	*pardus*	Kwalusi (baited)	6.1	29	4	0.12	(du Preez et al., 2014)
53	Zimbabwe	Africa	*pardus*	Mazunga (baited)	4.6	22	3	0.12	(du Preez et al., 2014)
54	Zimbabwe	Africa	*pardus*	Kwalusi (unbaited)	5.5	23	0	0	(du Preez et al., 2014)
55	Zimbabwe	Africa	*pardus*	Mazunga (unbaited)	2.8	17	0	0	(du Preez et al., 2014)
56	Kenya	Africa	*pardus*	Mpala Ranch, Laikipia	12.03	22	0	0	(O’Brien and Kinnaird, 2011)
57	Zambia	Africa	*pardus*	CD 2013, ESA	6.07	8	NA	NA	(Rosenblatt et al., 2016)
58	Zambia	Africa	*pardus*	HD 2013, ESA	4.2	5	NA	NA	(Rosenblatt et al., 2016)
59	Zambia	Africa	*pardus*	CD 2014, ESA	4.2	5	NA	NA	(Rosenblatt et al., 2016)
60	Zambia	Africa	*pardus*	HD 2014, ESA	5.87	7	NA	NA	(Rosenblatt et al., 2016)
61	Zambia	Africa	*pardus*	CD 2012, WSA	10.15	16	NA	NA	(Rosenblatt et al., 2016)
62	Zambia	Africa	*pardus*	CD 2013, WSA	8.3	10	NA	NA	(Rosenblatt et al., 2016)
63	Zambia	Africa	*pardus*	HD 2013, WSA	7.16	9	NA	NA	(Rosenblatt et al., 2016)

64	Zambia	Africa	*pardus*	CD 2014, WSA	7.16	9	NA	NA	(Rosenblatt et al., 2016)
65	Zambia	Africa	*pardus*	HD 2014, WSA	9.72	12	NA	NA	(Rosenblatt et al., 2016)
66	South Africa	Africa	*pardus*	Soutpansberg Mountains	10.41	10	4	0.29	(Grey et al., 2013)
67	Iran	Asia	*saxicolor*	Golestan	2.63	20	1	0.05	(Hamidi et al., 2014)
68	Iran	Asia	*saxicolor*	Bamou	1.87	6	1	0.14	(Ghoddousi et al., 2010)
69	Iran	Asia	*saxicolor*	Tandoureh	5.9	30	7	0.19	This study
70	Iran	Asia	*saxicolor*	Sarigol	8.02	10	1	0.09	This study
71	Iran	Asia	*saxicolor*	Salouk	4.01	12	2	0.19	This study
72	Armenia	Asia	*saxicolor*	Nuvadi	0.34	1	0	0	(Khorozyan et al., 2008)

Table 6.S2 Characteristics of candidate models generated in secr for Persian leopards based on two types of sampling, i.e. water-based versus trail-based camera trapping in Tandoureh National Park, northeastern Iran implemented in summer 2016. The models were fitted by maximising the full likelihood. g0=detection probability at trap location considered as home range center, D=density, sigm=spatial scale parameter, pmix=mixing parameter and EX=exponential detection function.

Model description	**Detection function**	**Paramet ers**	**Log likelihood**	**AIC**	**ΔAIC**	**AIC weight**
Trail-based sampling						
D~1 g0~1 sigma~h2 pmix~h2	EX	5	-385.0636	780.127	0	0.5430
D~1 g0~h2 sigma~h2 pmix~h2	EX	6	-384.9805	781.961	1.834	0.2170
D~1 g0~bk sigma~1 pmix~h2	EX	5	-386.1609	782.322	2.195	0.1812
D~1 g0~Bk sigma~1 pmix~h2	EX	5	-387.2875	784.575	4.448	0.0587
D~1 g0~h2 sigma~1 pmix~h2	EX	5	-390.2151	790.430	10.303	0.0000
D~1 g0~1 sigma~1 pmix~h2	EX	4	-393.1958	794.392	14.265	0.0000
D~1 g0~b sigma~1 pmix~h2	EX	5	-393.1749	796.350	16.223	0.0000
D~1 g0~t sigma~1 pmix~h2	EX	14	-387.0688	802.138	22.011	0.0000
Water-based sampling						
D~1 g0~bk sigma~1 pmix~h2	EX	5	-512.2915	1034.583	0	1
D~1 g0~b sigma~1 pmix~h2	EX	5	-520.5338	1051.068	16.485	0
D~1 g0~t sigma~1 pmix~h2	EX	14	-512.2328	1052.466	17.883	0
D~1 g0~Bk sigma~1 pmix~h2	EX	5	-523.9288	1057.858	23.275	0
D~1 g0~1 sigma~1 pmix~h2	EX	4	-529.0226	1066.045	31.462	0
D~1 g0~h2 sigma~1 pmix~h2	EX	5	-528.7358	1067.472	32.889	0
D~1 g0~1 sigma~h2 pmix~h2	EX	5	-528.7904	1067.581	32.998	0
D~1 g0~h2 sigma~h2 pmix~h2	EX	6	-528.7152	1069.430	34.847	0

Chapter 7:

Genetic variability and population partitioning of Persian leopard

Panthera pardus saxicolor in Iran

Genetic variability and population partitioning of Persian leopard in Iran

7.1. Abstract

Persian leopards *Panthera pardus saxicolor* have been extirpated from over 84% of their historic range, and now inhabit fragmented landscapes across Afghanistan through Iran to the Caucasus. Many populations are very low density. Understanding and maintaining genetic diversity and connectivity is important to prevent inbreeding and genetic drift, both of which can threaten population viability. Nonetheless large-scale genetic assessment has not been conducted to date. Here we used six highly polymorphic microsatellite loci and 555 base pairs of the NADH dehydrogenase subunit 5 (NADH-5) coding mitochondrial gene to describe the spatial genetic structure of Persian leopards in Iran, a country where most of the subspecies' population persists. Persian leopards exhibited moderately high genetic diversity at microsatellites (A_N = 7.45, H_O = 0.69, H_E =0.75) in comparison to other leopard populations globally. Mitochondrial genome sequencing revealed low haplotype diversity (Hd = 0.198) across three closely related haplotypes, with a common haplotype (89.5% of sampled individuals) distributed across most of Iran as well as the Caucasus and Turkmenistan. There was weak evidence of spatial population partitioning and no evidence for recent population expansion. The low mitogenome diversity could potentially be with evolutionary selective pressure on their mitochondria to adapt to severe oxidative stress and higher metabolic rates of their cold environments. We found none of the haplotypes previously reported in captive-born Persian leopards maintained in European zoos, highlighting the importance of careful genetic assessment of the captive population of Persian leopards.

Key words: Persian leopard, microsatellite, genetic variation, population structure, gene flow, NADH dehydrogenase subunit 5.

7.2. Introduction

As a large-bodied, wide-ranging felid, the leopard *Panthera pardus* has evolved to persist across a diverse range of habitat types (Macdonald et al., 2010). Adapting to extreme heterogeneity across its vast global range has resulted in remarkable morphological variation in leopards, encouraging taxonomists to describe upto 27 putative subspecies (Ellerman and Morrison-Scott, 1966; Herrington, 1986; Kitchener et al., 2017).

Leopards also show higher genetic diversity than many other big cats such lions *P. leo* (Dubach et al., 2005), tigers *P. tigris* (Luo et al., 2004), jaguars *P. onca* (Eizirik et al., 2001) and snow leopards *P. unica* (Janecka et al., 2017). Nonetheless, their heterozygosity is not spatially homogenous across their global diverse range of distribution (Uphyrkina et al., 2001), varying 2.5-fold between the most variable populations in Africa and India (Dutta et al., 2012a; Ropiquet et al., 2015) to the least variable populations observed in the Far East of Asia and the Arabian Peninsula (Perez et al., 2006; Uphyrkina et al., 2002).

West Asian leopards have been subjected to continuous controversy over their taxonomy, phylogeography, genetic partitioning and variability. A total of seven putative subspecies have been reported for the region (Herrington, 1986; Zukowsky, 1964), although many of them were recently identified as synonymous based on molecular analysis (Farhadinia et al., 2015a; Rozhnov et al., 2011; Uphyrkina et al., 2001). Currently, two subspecies *P. p. saxicolor*=*P. p. dathei*=*P. p. sindica*=*P. p. ciscaucasica*= *P. p. tulliana* and *P. p. nimr*=*P. p. jarvisi* are valid for West Asia (Kitchener et al., 2017). Unlike taxonomy and phylogeography which are moderately studied; our understanding of spatial population structuring and the genetic variability of West Asian leopards is poor. Previous analyses of intraspecific genetic variation of West Asian leopards have been limited by the application of only mitogenome sequencing (Farhadinia et al., 2015a), inadequate samples from the region (Rozhnov et al., 2011), and the use of captive-born individuals whose origin is not always certain (Uphyrkina et al., 2001) which raised controversy about the certainty of the geographic origin (Khorozyan et al., 2006). Here we attempt to address all three of these issues using both nuclear and mitochondrial genomes based on a spatially representative sample of Persian leopards in Iran, which hosts 86% of the subspecies extant range (Jacobson et al., 2016).

Leopards can exhibit rates of gene flow sufficiently high to limit the accumulation of genetic differences between subpopulations, even in human-altered landscapes (Dutta et al., 2013). They show significant population genetic structuring in fragmented areas (Dutta et al., 2012a; Ropiquet et al., 2015) whereas spatial partitioning is less evident in continuous landscapes (Miththapala et al., 1991; Spong et al., 2000). An increase in genetic structure combined with reduced gene flow can cause genetic drift and lead to diminished genetic diversity, which negatively impact short- and long-term persistence of wild populations, including large felids (Luo et al., 2004; Uphyrkina et al., 2002; Wultsch et al., 2016).

Microsatellite markers evolve faster than mitochondrial sequences, so they allow identification of recent reductions in gene flow mediated by anthropologic barriers (Avise, 2009). Mitochondrial DNA (mtDNA) markers also have been extensively used to reveal the stochastic processes of coalescence and lineage sorting of leopards (Farhadinia et al., 2015a; Ropiquet et al., 2015; Rozhnov et al., 2011; Uphyrkina et al., 2002, 2001). Within the mtDNA, the NADH dehydrogenase subunit 5 (NADH-5) coding gene has the highest variability known in the leopard mitochondria (Uphyrkina et al., 2001).

The primary concern of this research is to examine the population genetic structuring of leopards in Iran in regard to their current distribution which is impeded by several geographic barriers such as deserts or heavily populated areas (Ebrahimi et al., 2017). We also evaluated their genetic variability at both nuclear and mitochondrial sequences. With the increasing demand for leopard translocation in order to resolve the threat of severe livestock raiding (Farhadinia et al., 2015b; Weise et al., 2015) or to restore the historic range of the species (Breitenmoser et al., 2014; Rozhnov et al., 2011), our findings are helpful for guiding conservation interventions and avoiding compromising natural spatial genetic structure and diversity (Ropiquet et al., 2015).

7.3. Materials and Methods

7.3.1. Sample collection and DNA extraction

In total, 49 tissue samples, from both muscle and skin were obtained from wild-born dead leopards or individuals confiscated from poachers, all with known geographic origin in Iran. We also sampled four live leopards anesthetized for as part of a satellite telemetry project in northeastern Iran (Fig 7.1; permissions number 31/12630, 93/16270, 93/16258 and 93/16270 issued by the Iranian Department of Environment).

Samples were collected from three major regions of leopard range associated with major mountain chains, after suitability modelling by Ebrahimi et al. (2017). The sampled regions were West (Zagros mountains), North (Alborz mountains) and Northeast (Kopet Dag and Aladagh mountains). There are substantial natural (e.g. open areas) and anthropogenic barriers (e.g. high volume highways) between the three regions. The North and the Northeastern regions adjoin mountains in Afghanistan and Turkmenistan are less prominent.

Total DNA was extracted from leopard tissue samples using a standard phenol/chloroform method described in Sambrook et al. (1989), employing negative controls for each batch of extractions. No detectable DNA (as visualized on agarose gel) or polymerase chain reaction (PCR) product was obtained from any of the negative controls. Sample preparation and DNA extraction were conducted in a laminar flow hood in an area isolated from other samples to prevent any contamination.

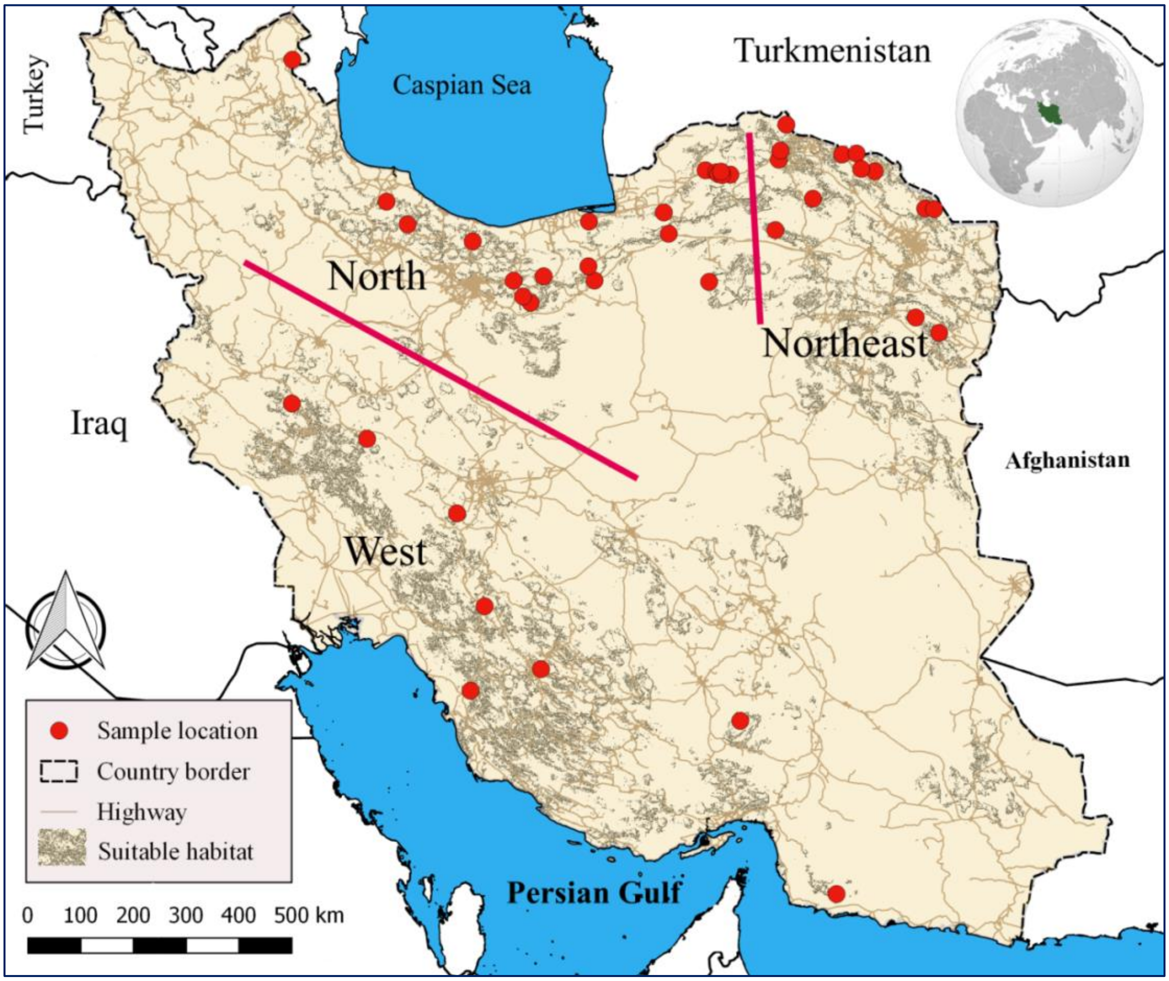

Fig 7.1 Location of leopard samples in Iran and spatial configuration of *a priori* geographic regions, based on the habitat suitability map, modelled by Ebrahimi et al. (2017). Red lines indicate barriers between geographic regions.

7.3.2. DNA sequencing and genotyping

7.4.2.1. Mitochondrial DNA

Genomic DNA was used for PCR amplification of mtDNA control region (CR) and the NADH-5 gene. The fragment of 600 bp of the central conserved portion of the mtDNA CR was amplified. We used two primers PAN-CCR-F (CTCAACTGTCCGAAAGTGCTT) and PANCCR-R (CCTGTGGAAGCAATAGGAATT), initially used for jaguars (Eizirik et al. 2001) which overlapped with 85 bp of the variable region directly adjacent to the central conserved region of the mitochondrial CR, already sequenced for leopards (Uphyrkina et al. 2001). We also amplified the fragment of 555 bp of the 5′ end of the NADH-5 mitochondrial gene in two separate pieces that overlapped over approximately 140 bp, following Uphyrkina et al. (2001). Two sets of primer pairs were combined as follows: F/RL2 (F: 5′-GTGCAACTCCAAATAAAAG-3′ and RL2: 5′-TAAACAGTTGGAACAGGTT-3′) and FL2/RL4 (FL2: 5′-CGTTACATGATCGATCATAG-3′, and RL4: 5′-TTAGGTTTTCGTGTTGGGT-3′). Apart from the forward primer F (from Johnson et al., 1998), all primers were designed from leopard sequences (Uphyrkina et al., 2001). A total of 26 leopards were used for amplification of CR while we expanded our sampling from a previous study using this marker (Farhadinia et al., 2015a) to achieve a sample size of 38 leopards.

For each reaction, 35 cycles were performed with 5 min initial denaturation at 95 °C followed by denaturation at 94 °C for 0.5 min, 1.5 min annealing at 50 °C, 1 min extension at 72 °C, followed by a 5 min final extension at 72 °C. Products were checked in 1.5% agarose gel in Tris–borate–EDTA (TBE) buffer. Purification was carried out using column-based purification kits (millipore) using a vacuum for filtering. The segment sequences were visualized using an ABI-3730 XL genetic analyzer (Applied Biosystems; http://www.appliedbiosystems.com).

7.4.2.2. Nuclear DNA

We used a panel of seven highly polymorphic microsatellites, *FCA672*, *E7*, *FCA008*, *F42*, *FCA126*, *FCA279* and *FCA304* which were screened out of 16 published microsatellite used for leopards (Dutta et al., 2012b). All loci were dinucleotides, except *F42* which was a tetra-nucleotide repeat. This panel has been shown to distinguish effectively spatial structure and migration among leopards in central India (Dutta et al., 2013, 2012a).

For all the microsatellite primer standardizations, amplification was carried out in 15 µl reaction volumes containing 7.5 µl Amplicon 2x master mix (Ampliqon, Odense M, Denmark), 0.8 µl of each primers (Macrogen, Rep. of Korea), and 100 ng of DNA extract in an Eppendorf thermocycler. PCR negatives were incorporated in all reaction setups to monitor contamination.

The PCR conditions included an initial denaturation (95°C for 5 min); 35 cycles of denaturation (94°C for 30 secs), annealing (61°C for 30 secs) and extension (72°C for 30 secs); followed by a final extension (72°C for 10 min). The PCR products were visualized in a 1.5% agarose gel and sequenced from both ends on an ABI377 capillary sequencer.

7.3.3. Data analysis

7.3.3.1. Mitochondrial DNA

We aligned sequences using the progressive pairwise alignment implemented in Geneious® 11.0.3 to a consensus length of 555 bp for NADH-5 and of 78 bp for CR with final corrections done by eye. The NADH-5 dataset consisted of 38 sequences from Iran and 97 reference leopard NADH-5 sequences from NCBI GenBank (Table 7.S1). For the CR dataset, we collated 22 leopard sequences from this study, 15 leopard CR reference sequences as well as 66 CR sequences from other felids from NCBI GenBank (Table 7.S2).

We carried out a statistical selection of best-fit models of nucleotide substitution based on Bayesian information criteria (BIC) in JModelTest 2.1.10 (Darriba et al., 2012), yielding HKY+I+G as the best nucleotide substitution model. We then constructed phylogenies using a Bayesian method for NADH-5 sequences. Phylogenies were built with MrBayes 3.2 (Ronquist and Huelsenbeck, 2003) using the evolutionary model to the GTR substitution model with gamma-distributed rate variation across sites and a proportion of invariable sites. We ran four simultaneous Monte Carlo Markov chains (MCMC) for 5,000,000 generations with a burn-in-length 200,000. The resulting trees were sampled every 1000 generations with a subsample frequency of 400. Tracer 1.5 (Rambaut and Drummond, 2009) was used to check for stabilization and convergence between runs.

The NADH-5 phylogenies were rooted with whole mitogenome sequences of African lion *P. leo* (AF385614.1 and KY996535), snow leopard *P. uncia* (EF551004.1), jaguar *P. onca* (KP202264.1 and KF483864.1) and tiger *P. tigris* (KF297576.1 and JF357972.1) obtained from NCBI GenBank. The haplotype network was drawn with the software tool PopART using a Median-joining network (Leigh and Bryant, 2015).

Genetic statistics (the number of haplotypes, polymorphic sites, haplotype diversity and nucleotide diversity) in Iran were compared to other areas in the world using DnaSP 6.10 (Librado and Rozas, 2009). We also implemented two neutrality tests under demographic changes, the Fu's F_S and Tajima's D (Fu, 1997) to test the hypothesis of selective neutrality and to detect past population growth (Ramírez-Soriano et al., 2008). Negative values suggest a recent population expansion resembling positive selection (or, under certain circumstances, negative selection), whereas positive values suggest recent bottlenecks or purifying selection.

7.3.3.2. Nuclear DNA

Microsatellite alleles were scored using Geneious® 11.0.3 software (Biomatters Ltd., Auckland, New Zealand). We used MICRO-CHECKER 2.2.3 to detect loci containing errors due to null alleles, stuttering and large allele dropout (Van Oosterhout et al., 2004). Standard estimates of genetic diversity were derived from GENALEX 6.5 (Peakall and Smouse, 2006) including the number of alleles (A_N), number of private alleles (A_P), effective number of alleles (A_E), observed heterozygosity (H_O), expected heterozygosity (H_E), fixation index (F_I, I - (H_O/H_E) and probability of identity (P_{ID}). Loci were tested for deviations from Hardy-Weinberg equilibrium with significant P-values adjusted for multiple comparisons. In order to examine if the mean H_O is significantly lower than the mean H_E, a t-test was used after checking for homogeneity of variances using the Bartlett test. We also used the statistical technique of rarefaction that accommodates the effect of small sample size for calculating unbiased allelic richness, i.e. the number of alleles in a sample using HP-RARE 1.0 (Kalinowski, 2005).

We estimated N_e using the molecular co-ancestry method of Nomura (2008), as implemented in NeEstimator V2 (Do et al., 2014) using the linkage disequilibrium method. In order to facilitate evaluation of the effects of low frequency alleles on estimates of effective size, we used a critical value of 0.014 (equal to 1/(2S), where S is the number of individuals with data at both pairs of loci). The confidence intervals around estimates were calculated using the jackknife (i.e. non-parametric) method. Then, we used two ratios of 0.11 based on different taxa (Frankham, 1995) and 0.42 calculated from felid case studies (Spong et al., 2000) between census and effective population sizes (N/N_e) to project current population size of leopards.

We also estimated the pairwise kinship coefficient F_{ij} in GENALEX 6.5, defined as the probability that genes share ancestry, or are ‘identical-by-descent’ (Ritland, 2000). This method is less sensitive to small-sample biases and distributional assumptions than other methods (Ritland, 1996). All these analyses were carried out in GENALEX 6.5 (Peakall and Smouse, 2006).

We analysed the statistical power of the panel of microsatellite loci to detect structure among sampling regions of leopards in Iran at different F_{ST} levels using the simulation-based program POWSIM (Ryman and Palm, 2006). It takes into account the sample sizes used and the variability of the loci. Power is expressed as the proportion of significant outcomes of Fisher's exact test statistic after 500 replicates and 0.80 was considered as the minimum acceptable power level.

We assessed population structure using several methods. We first estimated the F_{ST} index (Weir and Cockerham, 1984) as a standardized measure of the genetic variance among predefined groups (North, Northeast and West). It is most suited for inferences of the influence of demographic processes such as genetic drift and gene flow on genetic population structure (Meirmans and Hedrick, 2011). We also estimated Analysis of Molecular Variance (AMOVA) for the hierarchical analysis of the genetic diversity in a set of sampled populations based on variance components from a matrix of pairwise squared Euclidean distances between individuals (Excoffier et al., 1992). The number of migrants successfully entering a population per generation (N_m) was also calculated.

We also performed population assignment tests with frequency methods to evaluate the level of differentiation in GENALEX 6.5. These tests yield log-likelihood for each individual to predict its population, based on its genotype and allele frequencies. If assignment probability was highest for a population (or region) in which that individual was not sampled, it was considered genetically mis-assigned.

We then performed a Bayesian model-based clustering method in the program STRUCTURE 2.3 (Pritchard et al., 2000) to assess the level of genetic stratification in our multi-locus data set. We explored the number of genetic clusters (*K*) within our samples and estimated the ancestry for each individual (Pritchard et al., 2000). We assumed the population admixture models and allele frequencies correlated. STRUCTURE uses a MCMC procedure to estimate the posterior probability that the data fit the hypothesis of *K* clusters [(Pr (X|*K*)]. We varied the number of potential genetic clusters from 1 to 7 and performed 10 independent runs for each value of *K* using a burn-in of 1,000,000 MCMC steps followed by an additional 500,000 iterations. We used an ad hoc statistic Δ*K* based on the second order rate of change in the log probability of data between successive *K* values to detect the number of genetic clusters of individuals (Evanno et al., 2005), calculated using the STRUCTURE HARVESTER online program (Earl, 2012).

In order to infer subpopulation structure and cluster assignment, we carried out a Discriminant Analysis of Principal Components (DAPC) using ‘adegenet’ package (Jombart, 2008) in the *R* environment for statistical computing (R Development Core Team, 2013). It provides an efficient description of genetic clusters using synthetic variables, the discriminant functions, which show differences between groups as best as possible while minimizing variation within clusters (Jombart and Collins, 2015). We first performed a Principal Component Analysis (PCA) to identify directions of maximal variance. Then the most informative principal components were retained. We then ran a *K*-means clustering on the data, to maximize the variation between *K* groups, by incrementally increasing *K*. The most likely *K* is then identified based on a Bayesian Information Criterion (BIC) by comparing different *K*-means clustering solutions.

Then a discriminant analysis was performed on these data (retained PCs which explain the most variance, and clusters which have maximum between cluster variance, but minimum within cluster variance). We specified the maximum number of clusters as $K = 7$. DAPC also provides membership probabilities for each individual to each identified group (or subpopulation).

We hypothesized that there is ‘Isolation-by-Distance’, meaning a positive relationship between geographic and genetic distance between sampled individuals. We performed a Mantel test for Matrix Correspondence in GENALEX 6.5, yielding a correlation coefficient for the two data matrices, with a range from –1 to +1, with a test for a significant relationship by random permutation. Geographic distances among individuals were calculated using natural logarithmic conversion of pairwise linear Euclidian distances between individuals. Significance was determined using 999 permutations.

7.4. Results

7.4.1. Mitochondrial DNA

We obtained a total of 38 NADH-5 sequences for sampled individuals and also used samples described in a previous study (Farhadinia et al., 2015a). Likewise, we extracted and sequenced CR successfully from 22 individuals (four additional samples failed in genotyping). A total of 550 bp (NADH-5) and 78 bp (CR) were recovered, corresponding to position numbers 12781–13330 and 16818-16895, respectively (nucleotide numbers from the reference Panthera pardus sequence – GenBank accession number EF551002.1; Lei et al., 2011). Sequenced individuals spanned a large part of the leopard’s range in Iran (Fig 7.2).

Our CR samples had at least two nucleotides difference from other published leopard CR haplotypes (Uphyrkina et al., 2001), whereas they had no difference in nucleotide sequence with the tiger nuclear copy of mitochondrial CR (accession number AF053056; Cracraft et al., 1998) (Table 7.S2). The hypothesis that these anomalous CR sequences are nuclear is strengthened by a phylogenetic analysis, showing a strongly supported relationship between the putative leopard nuclear CR sequences and the tiger nuclear copy of mitochondrial CR, but not between our CR sequences and previously published sets of CR haplotypes (Uphyrkina et al., 2001).

Surprisingly, the pairwise identity between our CR sequences and other leopard mitochondrial sequences in GenBank was lower (96.2%) than with tiger (98.6%) or snow leopard (97.4%). Similarly, the percentage of identity was the highest (100%) when our CR sequences were aligned with the tiger nuclear copy of mitochondrial CR (accession number AF053056; Cracraft et al., 1998). The available mitochondrial control region from West Asian leopards (accession numbers AY035243 to AY035245; Uphyrkina et al., 2001) had lower similarity (94.9%) with our CR sequences. Thus, we excluded the CR sequences from our analysis.

Our samples of the NADH-5 gene samples were grouped into three closely related haplotypes: IRAN1 (N = 34, 89.5%), IRAN2 (N = 1, 2.6%), and IRAN3 (N = 3, 7.9%), differing from one another only in one substitution (Fig 7.3). IRAN1 and IRAN2 were identical to the published haplotypes az2 (N = 9) and ir2 (N = 2), respectively, from the Alborz range, Turkmenistan and the Caucasus (Rozhnov et al., 2011). The haplotype IRAN1 was dominant in our sample, commonly occurring throughout Iran. One individual belonging to the haplotype IRAN2 was from the Alborz while three individuals assigned to haplotype IRAN3 were from the southernmost Zagros (Fig 7.2).

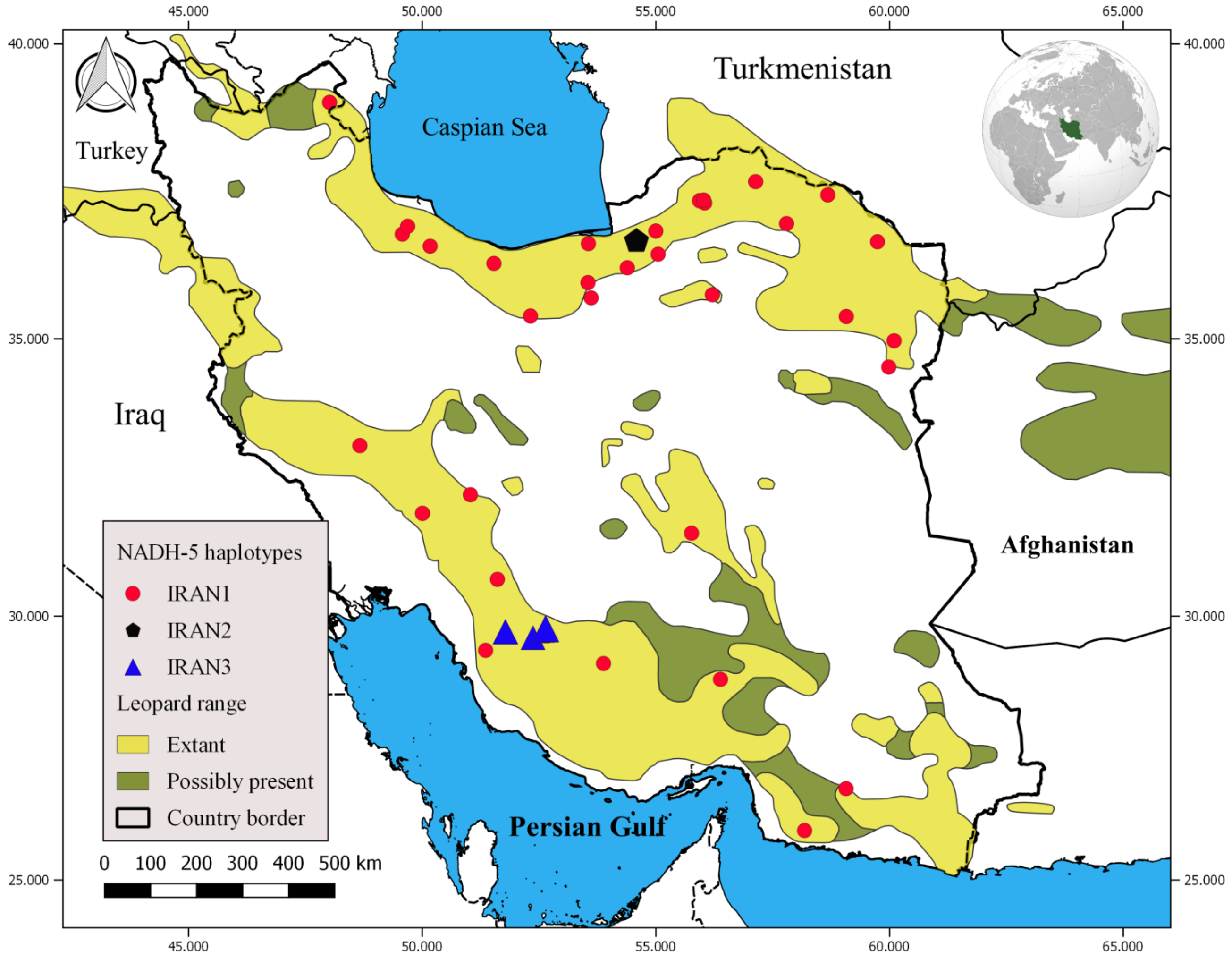

Fig 7.2 Locations of Persian leopard samples sequenced for mtDNA analysis. The map shows the distribution of the three mtDNA haplotypes found in Iran: IRAN1 (N=35), IRAN2 (N=1) and IRAN3 (N=3).

The MCMC chain produced in MrBayes was long enough for convergence and there were large intervals between the posterior samples to avoid autocorrelation (effective sample size as the number of effectively independent draws from the posterior was ≥ 572). The Bayesian phylogenetic analysis showed that all Iranian leopards together with leopards from Central Asia, East Asia, Indonesia and the Indian Subcontinent form a monophyletic clade in relation to an

African and South Arabian clade (Fig 7.3). Within this clade, leopards from Iran represent a monophyletic subclade (with posterior probability 0.86). A median-joining network of mtDNA haplotypes further illustrated the patterns observed in the Bayesian phylogenetic tree, suggesting the divergence of the Iranian female lineage from Asian leopards between 16 393-270 492 years BP (95% highest posterior density) and the polyphyletic kinship between the Asian and Afro-Arabian leopards (Fig 7.4).

Table 7.1 summarizes the molecular diversity of the mtDNA gene segment sequences that we used in our analyses. The estimated haplotype diversity in Persian leopards was low when compared to the other clades (*Hd* = 0.198; Table 7.2). There was no evidence of significant negative values for Iran's leopards based on Fu's F_S test (-0.485, *P*=0.144, Table 7.2), implying that Iran's population has not experienced any recent demographic expansion.

Table 7.1 Molecular diversity indices of mtDNA gene segment (NADH-5, 550 bp) in *Panthera pardus* (*P. p. nimr* was excluded from subdivision analysis due to a sample size of one individual).

Number of	**Iran**	**Asia**	**Africa**	**All individuals**
Gene copies	38	64	15	117
Unique haplotypes	3	19	11	34
Substitution sites	2	25	26	50
Private substitution sites	1	12	14	50
Transition sites	2	25	23	48
Transversion sites	0	0	3	4
Substitutions	2	25	26	52
Transitions	2	25	23	48
Transversions	0	0	3	4
Indels	0	0	0	0

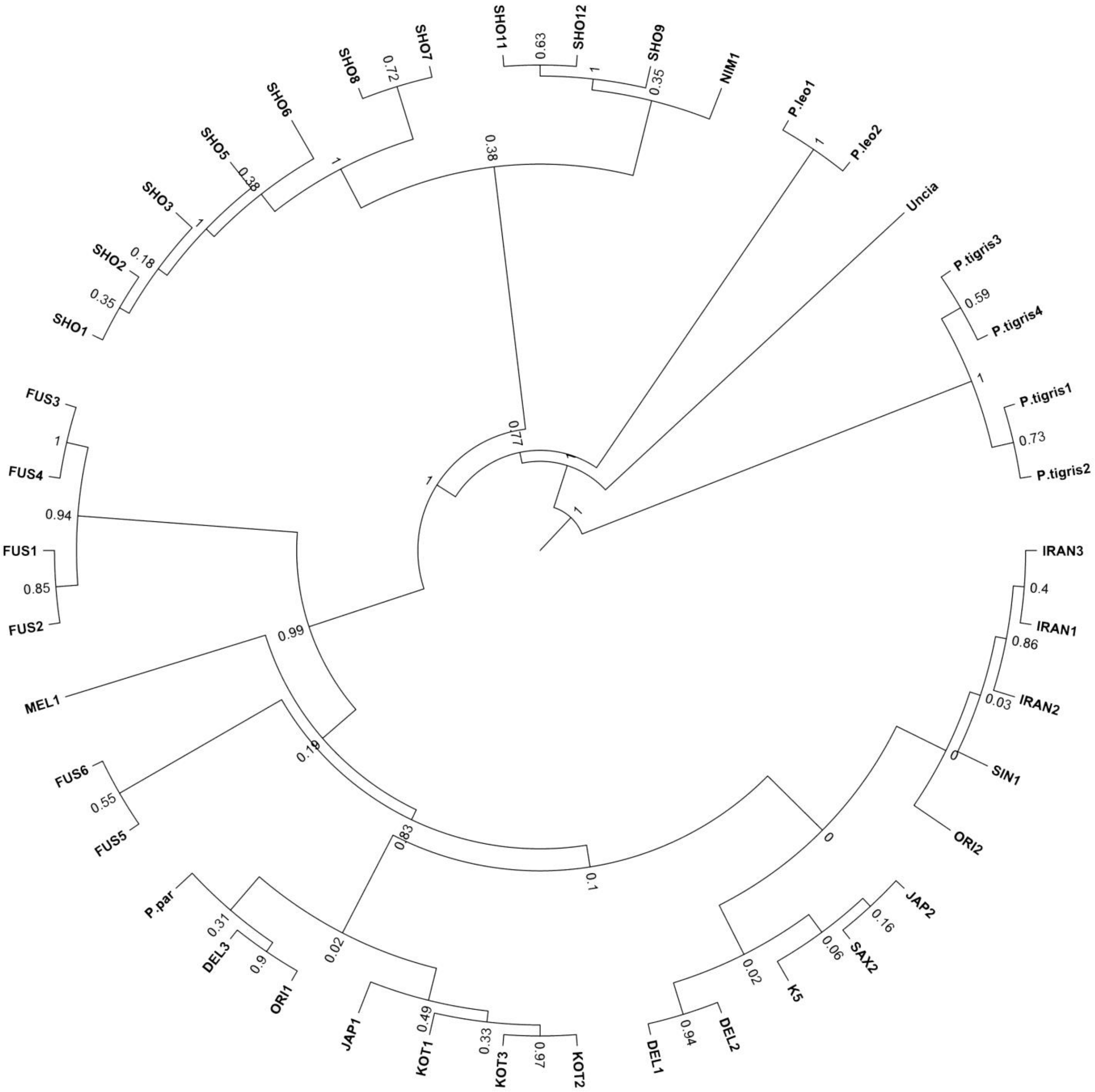

Fig 7.3 Bayesian phylogeny based on 555 bp for NADH-5 of leopard subspecies and other *Panthera* species. Bayesian posterior probability values are shown at nodes. Values at nodes are posterior probabilities. IRAN1, IRAN2 and IRAN3 are haplotypes from all over Iran, from the Alborz and from the southernmost Zagros, respectively.

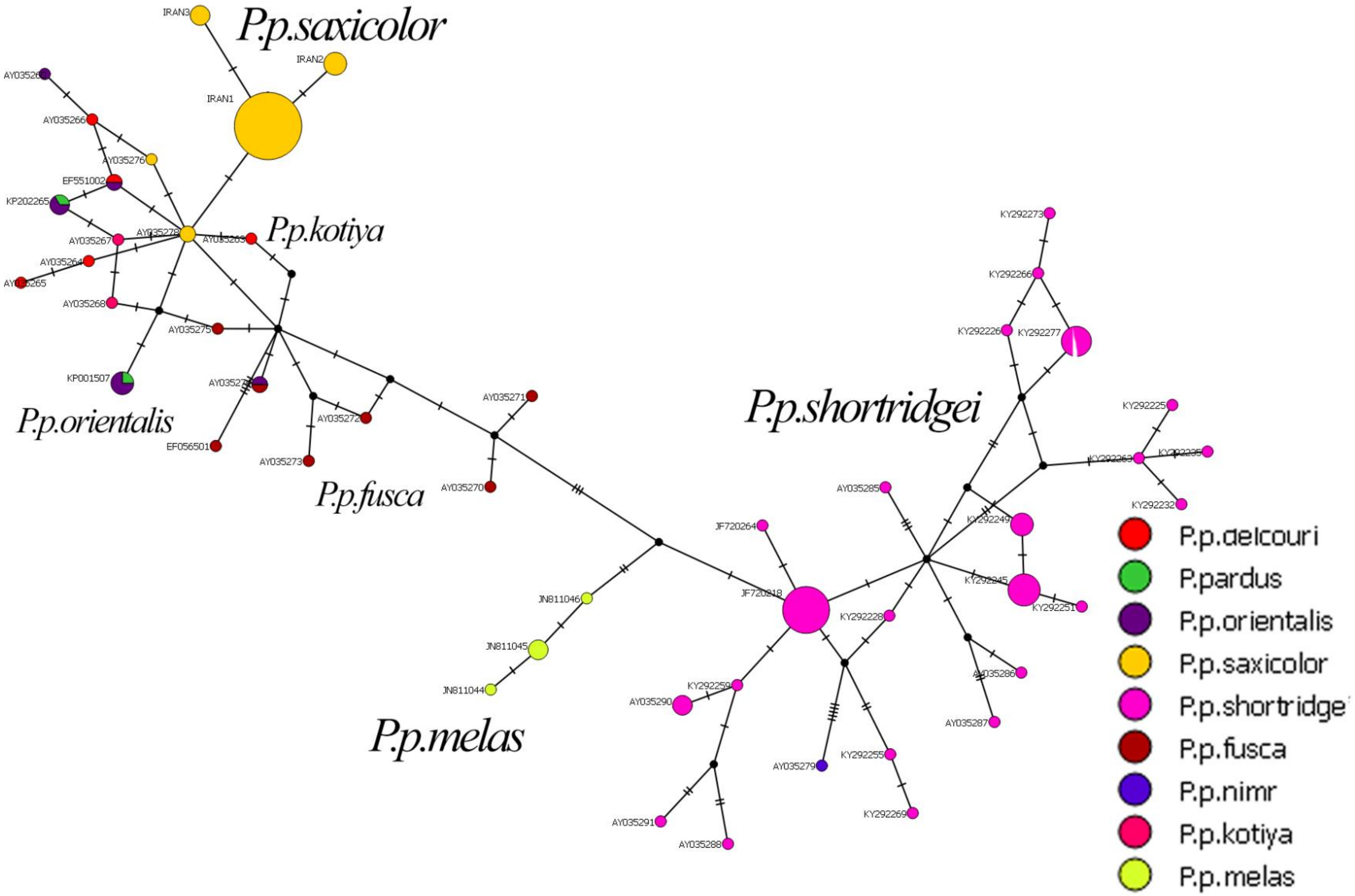

Fig 7.4 Median-joining networks based on 555 bp for NADH-5 of *Panthera* species. Persian leopards (haplotypes IRAN1, IRAN2 and IRAN3; orange) obtained based on samples from Iran form a monophyletic clade which is at least one mutation away from other Persian leopard haplotypes reported by Uphyrkina et al. (2001).

Table 7.2 Genetic statistics for Persian leopards compared with other leopard subspecies based on the 555 bp mtDNA NADH-5. N, number of individuals; *h*, number of haplotypes; *Hd*, haplotype diversity; SD, standard deviation; *Pi*, nucleotide diversity (per site); *K*, average number of nucleotide differences; *P*, variable (polymorphic) sites. Asterisk indicates significance values ($P<0.05$) for the hypothesis of departures of DNA sequence variability from the expectations of selective neutrality.

Group	**N**	***h***	***Hd* (SD)**	***Pi* (SD)**	***K***	***P***	**Fu's F_S**	**Tajima's *D***
Iran	38	3	0.198 (0.083)	0.00037 (0.00016)	0.202	2	-1.485	-1.1020
Asia	73	23	0.724 (0.058)	0.00463 (0.00076)	2.556	35	-13.386*	-2.067*
Africa	145	22	0.874 (0.013)	0.01645 (0.00036)	9.065	40	1.911	0.775

7.4.2. Nuclear DNA

A total of 41 representative individuals were genotyped at seven microsatellite loci, but we excluded locus *FCA279* due to lack of polymorphism among genotyped individuals. Among the six markers, no evidence for scoring error due to stuttering, large allele dropout or null alleles were found by MICRO-CHECKER, therefore we did not discard any loci from further analyses.

Standard estimates of genetic diversity were consistently high across the sampled range of Persian leopards (A_N = 6.67-8.17, A_E = 4.54-5.22, H_O = 0.66-0.71, H_E =0.75-0.77; Table 7.3). The number of private alleles expected in a sample of each geographic region after accounting for sample size was low (ranging 0.98 to 1.40; Table 7.3). All the microsatellite loci were polymorphic with the mean number of alleles per locus as 10.67 (± SE 2.0), varied from 5 in *FCA672* to 16 in *FCA304* (Table 7.4).

Table 7.3 Estimates of genetic diversity for 41 Persian leopards genotyped at six microsatellites. *n*, sample size; $A_{N,}$ the number of alleles; A_{PR}, allelic richness; $A_{P,}$ number of private alleles; A_E, effective number of alleles ($1/\sum p^2$); A_{PR}, private allelic richness; *I*, information index; $H_{O,}$ observed heterozygosity; H_E, expected heterozygosity; F_I, fixation index ($1 - H_O/H_E$); P_I, probability of identity for six loci. * $\boldsymbol{A_R}$ and $\boldsymbol{A_{PR}}$ calculated by HP-RARE 1.0 software rarefied to sample of 6 genes.

Geographic region	**n**	A_N	A_R*	A_P	A_E	A_{PR}*	*I*	H_O	H_E	F_I	P_{ID}
North	19	8.167	5.03	1.500	5.224	0.98	1.723	0.660	0.767	0.140	1.5E-07
Northeast	14	7.500	5.10	1.500	4.536	1.18	1.668	0.712	0.748	0.040	3.2E-07
West	8	6.667	5.48	0.667	5.044	1.40	1.639	0.723	0.745	0.039	2.3E-07
Iran	41	7.45	5.20	NA	5.735	1.20	1.894	0.69	0.753	0.073	NA

Table 7.4 Genetic variability of 41 Persian leopards at six microsatellites.

Locus	**Size range**	**Number of alleles**	A_E	*I*	H_O	H_E	F_I	P_{ID}
FCA672	103-117	5	3.042	1.271	0.538	0.671	0.198	2.4E-01
FCA126	151-165	8	4.180	1.622	0.703	0.761	0.076	1.3E-01
F42	250-288	13	5.377	2.041	0.647	0.814	0.205	7.1E-02
E7	168-196	16	7.656	2.296	0.725	0.869	0.166	4.5E-02
FCA008	150-160	6	5.071	1.703	0.742	0.803	0.076	1.1E-01
FCA304	105-145	16	9.086	2.431	0.780	0.890	0.123	3.6E-02
Overall mean (SE)		10.67 (2.0)	5.74 (0.9)	1.89 (0.2)	0.69 (0.03)	0.80 (0.03)	0.14 (0.02)	1.0E-01

H_O was significantly lower than H_E (t = 6.130, df = 5, $P>0.001$). Chi-square tests showed significant deviation from Hardy-Weinberg Equilibrium in three loci, i.e. *FCA126*, *F42* and *FCA304* ($P<0.05$). The pairwise kinship coefficient F_{ij} was on average -0.015 (± SD 0.064, range: -0.116-0.320), showing that our sampled population was generally unrelated. N_e (CI 95%) was estimated at 68.6 (34.2-348.0) and consequently, census population size was estimated at 162 and 624 individuals for two different scenarios.

Power analysis showed that the probability of detecting a F_{ST} more than 0.02 is more than 80% (Table 7.5). Intra-population molecular variance accounted for 95% of total molecular variance. Pairwise F_{ST} comparisons between sampled geographic regions (Table 7.6) suggested a panmixis pattern between the North and other two neighbouring regions.

Table 7.5 Microsatellite power analysis. Probability of microsatellite loci detecting true F_{ST} values according to Fisher’s exact test and Chi-Squared tests analyzed in Powsim software with an effective population size (N_e) of 1000.

F_{ST}	Fisher' Exact Test	Chi-Squared Test
0.001	0.0820	0.0520
0.0025	0.128	0.1100
0.005	0.1940	0.1720
0.0100	0.4840	0.4520
0.0150	0.7140	0.7160
0.0200	0.8840	0.8840
0.025	0.9520	0.9540
0.050	1.0000	1.0000

Table 7.6 Pairwise *AMOVA* F_{ST} (below the diagonal) and N_m estimates (above the diagonal) among three geographic regions for Persian leopards in Iran as a tri-matrix. Asterisk indicates significance for F_{ST} values ($P<0.05$).

Geographic region	**North**	**Northeast**	**West**
North		4.1	3.5
Northeast	0.057*		16.6
West	0.067*	0.015	

The PCA did not reveal groups consistent with the geographic distribution of sampled localities. The first and second PCs, each corresponded with a small inertia (7.7% and 7.3%, respectively). We retained the first 20 PCs, explaining 0.83 of the total inertia to extract discriminant functions. Although three clusters were clearly discriminated using DAPC (Fig 7.6); nevertheless, there was no strong support for any of the top scoring cluster sizes based on their ΔBIC (**Error! eference source not found.**5 and Table 7.7). Group assignment in GENALEX produced confounding results for the two highest ranking *K* without any biologically meaningful spatial patterns. For example, samples from Golestan (n = 6) or Tandoureh (n = 4) National Parks never shared a single cluster in different assignment scenarios.

Delta *K* did not vary considerably between different numbers of clusters, spiking slightly in 7.70 for *K*=4. Nonetheless, the mean LnP(*K*) for different *K* had overlapping SD and the bar plots constructed in the STRUCTURE showed an even distribution of the alleles shared between different clusters (Fig 7.7), without any strong support for group assignments in all scenarios.

The Mantel test showed that the spatial structure was not strong (*Rxy*=-0.016 *P*=0.42; Fig 7.8), so that only 0.0002 (i.e. 0.016^2) of the genetic divergence was explained by geographic distance, indicating that more spatially separated pairs were not more different genetically.

Table 7.7 The strength of the evidence against the model with the higher BIC value for each cluster size (*K*) estimated in Discriminant Analysis of Principal Components (DAPC).

K	**BIC**	**ΔBIC**
3	34.6895	0
2	34.8117	0.12218
4	35.2944	0.60493
5	35.9389	1.24941
6	36.5376	1.84813
1	37.054	2.36453
7	37.0649	2.3754
8	37.4074	2.71794
9	38.4279	3.73846
10	39.4237	4.73419

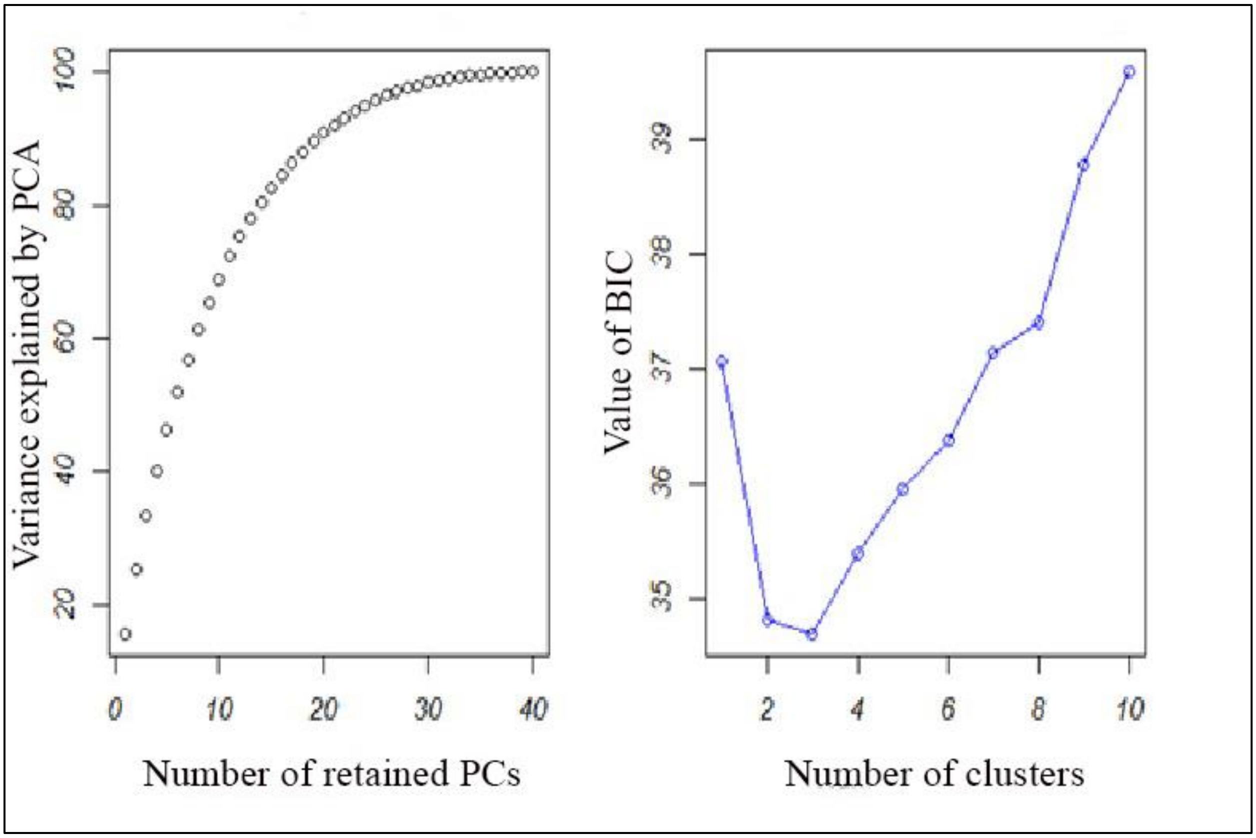

Fig 7.5 Cumulated variance explained by the eigenvalues of the PCA and a graph of BIC values for increasing values of *K* This graph shows a decrease of BIC until *K*= 3 clusters, after which BIC increases, but with weak support of ΔBIC (Table 7.6).

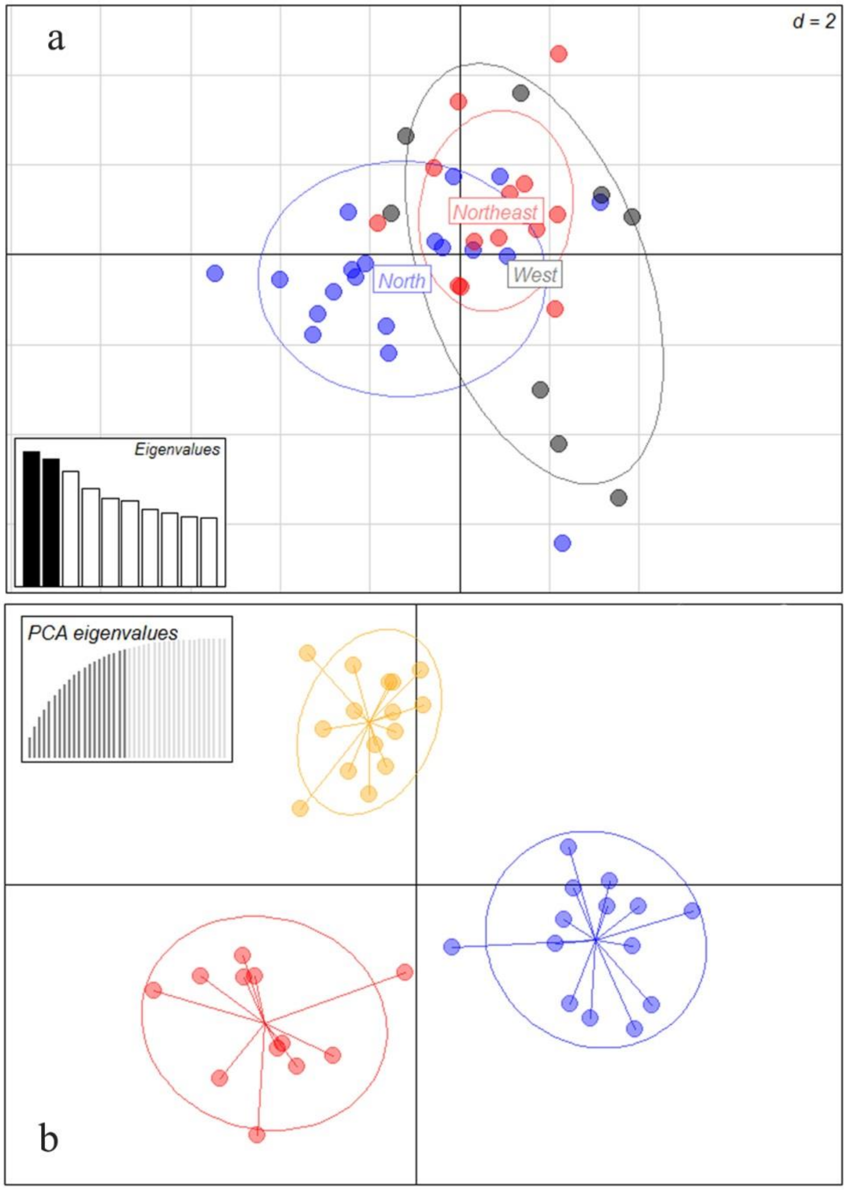

Fig 7.6 Scatterplot of PCA (a) and DAPC (b) for nuclear DNA loci of 41 leopard samples from Iran. Each point represents individual genotypes sampled across different geographic regions. Figure 7.6a shows the principal components 1 and 2 of the three *a priori* clusters of leopards in Iran and 95% inertia ellipses. Figure 7.6b shows a DAPC scatterplot of the density of individuals on the factorial plane. The ellipses and their centres are shown for three clusters. The graph of PCA eigenvalues retained in a dimension-reduction step (retained eigenvalues in black) is also illustrated as an inset in b.

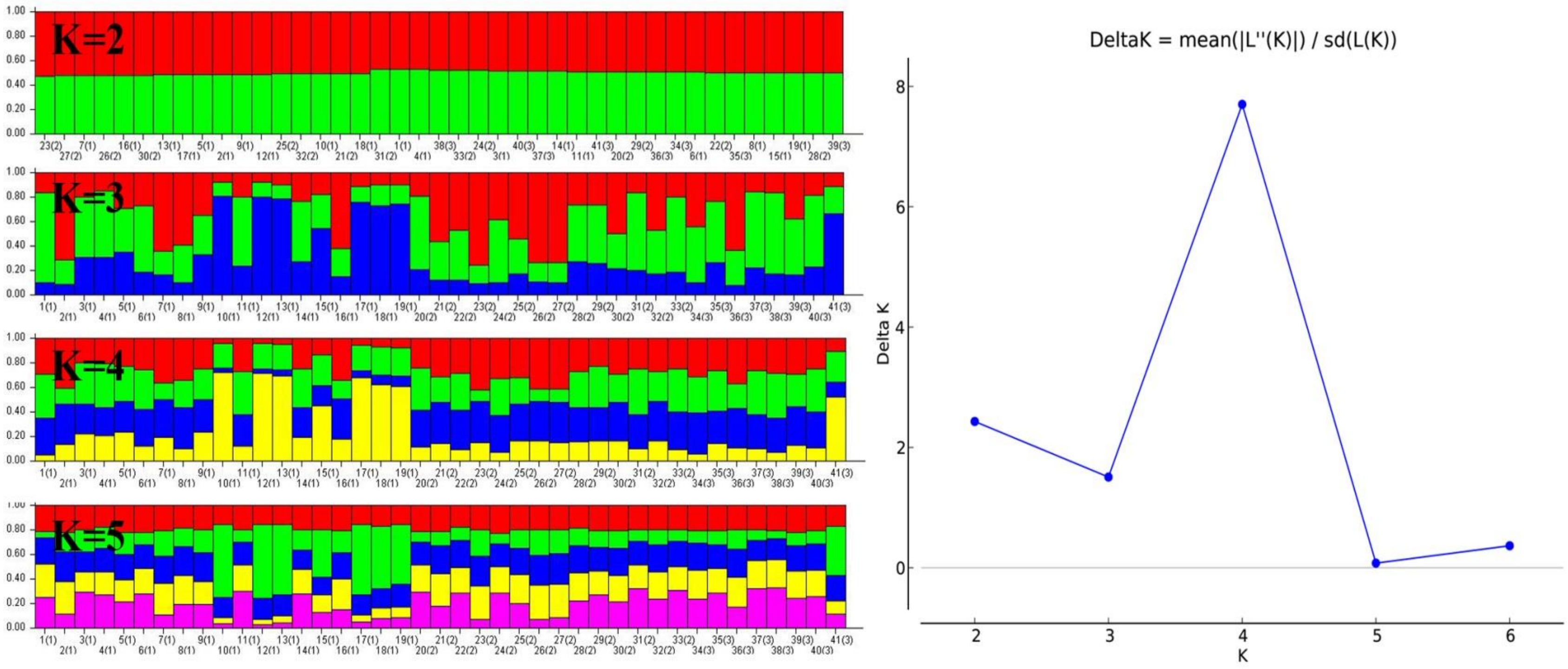

Fig 7.7 Graphical methods allowing detection of the true number of clusters *K*. Δ*K* calculated as Δ*K* = mean|L″(*K*)|/s[L(*K*)] for each suggested *K*. Bar plots constructed in STRUCTURE showing three admixed genetic clusters. Each individual is represented by a vertical bar, and the colored length of each bar indicates the probability of membership in each cluster.

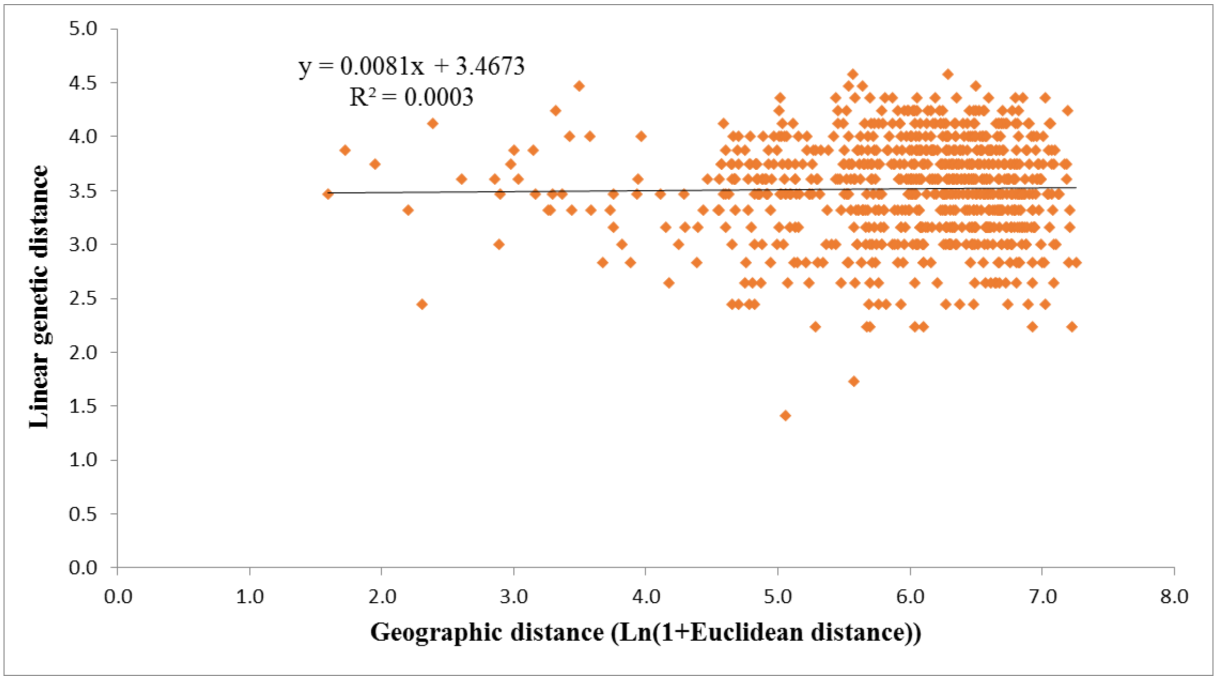

Fig 7.8 Mantel test for Isolation-by-Distance to investigate the occurrence of a positive correlation (*Rxy*>0) between pairwise linear genetic and geographic distance among 41 genotyped leopard individuals in Iran. The genetic and geographic distance were not correlated (*Rxy*=-0.016 *P*=0.42).

7.5. Discussion

We found contrasting results using the two types of markers; i.e. low mitogenome diversity and moderately high nuclear heterozygosity. Nonetheless, they both supported the lack of substantial population subdivision in Iran's leopards. However, the small number of microsatellite loci analysed requires us to view our findings as suggestive rather than conclusive.

7.5.1. Population partitioning

Our data supported a common maternal genealogy for leopards in West Asia and the Caucasus (Farhadinia et al., 2015a; Rozhnov et al., 2011) without evidence of demographic expansion. The common haplotype (IRAN1) was found in mountains separated by as much as 1,800 km in West Asia (Rozhnov et al., 2011).

The weak population subdivisions based on nuclear genealogies along with the unstructured matrilines suggest that Iran's leopards currently form a single gene pool. Although isolation by distance was not evident in Persian leopards, it has different effects on gene flow in various leopard populations. No evidence of isolation by distance was found between leopard populations along a gradient of almost 1400 km of Tanzanian habitats or for several patches of landscapes spread over 500 km in central India based on 18 and 7 microsatellites, respectively (Dutta et al., 2012a; Spong et al., 2000). Conversely, it was found to be the underlying mechanism affecting population structure across over 3000 km of leopard range in southern Africa based on eight microsatellites (Ropiquet et al., 2015).

The high dispersal capacity of leopards (i.e. making isolation less likely) makes them resilient to isolation. Persian leopards are known to range over long distances, up-to 170 km in West Asia (Farhadinia et al., 2017; E. Askerov pers.comm.). Nonetheless, observational data show no evidence of contemporary leopard mobility between Zagros (West) and the other two ranges (Ebrahimi et al., 2017). It is reasonable to assume that the N_m value derived is explained by dispersal events under historic conditions, rather than by current circumstances (Eizirik et al., 2001).

7.5.2. Genetic variability

Persian leopards have low mitogenomic diversity, similar to snow leopards, another *Panthera* species roaming Asia's rugged mountains (Janecka et al., 2017). In contrast, other sympatric large-bodied, wide-ranging carnivores such as brown bear *Ursus arctos* and Himalayan grey wolf *Canis himalayensis* show remarkable haplotype diversity (Ashrafzadeh et al., 2016; Murtskhvaladze et al., 2010; Werhahn et al., 2017). Felids, especially *Panthera* species, have the most recent divergence time and consequently a lower molecular evolution rate compared to Ursids and Canids (Wayne et al., 1991). This has resulted in little intra-specific genetic diversity and shorter inter-specific genetic distances (Kim et al., 2016; Slattery et al., 2000).

We acknowledge the low number of microsatellite loci investigated can be problematical for the analysis of leopard genetic structure (Matthee and Ropiquet, 2016; McManus and Smuts, 2016). Nonetheless, the current panel of highly polymorphic loci has high statistical power to detect F_{ST} value as low as 0.02. and has previously clarified fine-scale genetic partitioning among leopards in central India (Dutta et al., 2012a). It also enabled us to obtain an allelic richness per locus of 2.5 times more than previous analysis with 25 loci on Persian leopards (Uphyrkina et al., 2001).

7.5.3. Evolutionary perspective

The mitochondrial and nuclear genomes of leopards are highly variable across most of their global range (Anco et al., 2017; Ropiquet et al., 2015; Uphyrkina et al., 2001). Therefore, our findings in Persian leopards, i.e. low haplotype diversity and moderately high nuclear heterozygosity must be interpreted within the spatial context of Iran, and West Asia.

There are two possible explanations. From the demographic perspective, this scenario can be inherited from bottleneck events in recent or historic times (Ellegren and Galtier, 2016), similar to Amur tiger *P. t. altaica* (Russello et al., 2004), Amur leopards *P. p. orientalis* (Uphyrkina et al., 2002), Asiatic cheetah *Acinonyx jubatus venaticus* (Charruau et al., 2011) and Scandinavian lynx *Lynx lynx* (Hellborg et al., 2002). All these have experienced a recent or ongoing demographic bottleneck with a small founding population. Although Persian leopards survived widespread eradication attempts in the Caucasus during the 1900s, leaving a few dozen (Khorozyan and Abramov, 2007), no contemporary demographic bottleneck is known from Iran, with an estimated current population of between 550 and 850 individuals and wide distribution by the end of the 1900s (Kiabi et al., 2002). Importantly, when leopard populations collapse substantially, moderate mitogenomic variation is still expected (Wilting et al., 2016). It is therefore less likely that demography can comprehensively explain the current genetic pattern in Persian leopards.

Alternatively, it might be associated with the selective pressure on endothermic vertebrates to cope with the combined effects of hypoxia and cold stresses at high altitudes (Cheviron and Brumfield, 2012). There, they need adaptive evolution of the mitochondrial oxidative phosphorylation system to regulate oxygen usage and energy metabolism (da Fonseca et al., 2008; Hassanin et al., 2009). Janecka et al. (2017) suggested that the snow leopard mitogenome may have undergone selective pressure in hypoxic environments, resulting in remarkably low mitochondrial diversity.

Nonetheless, hypoxia should be less influential than hypothermia for the evolution of common leopards, because they are predominantly found at lower elevations. Similarly, the Siberian tiger, a subspecies persisting exclusively in cold environments has the lowest mitochondrial diversity of the tiger subspecies while exhibiting moderate nuclear heterozygosity (Luo et al., 2004). We suggest that with a high level of nuclear variability, Persian leopards may have experienced selective pressure in their mitochondria to adapt to severe oxidative stress and higher metabolic rates of their cold environments.

One potential source of mitochondrial discordance based on CR sequences is the amplification and sequencing of nuclear mitochondrial pseudogenes (numt) (Antunes et al., 2007). As the transposed mtDNA in the nuclear genome, numt indicates ancient hybridization among felids, particularly the genus *Panthera* (Li et al., 2016). It also has a decreased rate of evolution compared to that of mitochondrial sequencing and has been shown not to differentiate tiger subspecies (Zhang et al., 2006). Occurrence of numt in leopard mitogenome needs to be investigated further.

7.5.4. Management implications

We are unable to evaluate the demographic trend of leopards using the current census population size (162 to 624 individuals) for Iran and expert estimate of between 550 and 850 individuals (Kiabi et al., 2002). Nonetheless, based on systematic population surveys in six key Iranian reserves, an excess of 100 adult leopards have been detected (see chapter 6), suggesting that 162 is an underestimate. Our estimate of N_e must be interpreted with caution, because of the limited number of markers. Equally important, the linkage disequilibrium method is biased downward when the sample size is smaller than the N_e (England et al., 2006).

None of the haplotypes obtained in zoo-born Persian leopards (Uphyrkina et al., 2001) were detected either in our samples or in other wild-caught samples from West Asia and the Caucasus (Rozhnov et al., 2011). Although genetic differentiation between *ex situ* (SAX1, SAX2 and SIN1) and *in situ* haplotypes (IRAN1=az2, IRAN2=ir2 and IRAN3) are minor (2-4 mutations), it raises concern about the origin of captive-born leopards. Nuclear DNA can help to resolve the discrepancy, nonetheless it may be hard to achieve due to the need to standardize microsatellite profiles across laboratories.

Although the current genetic variability of Persian leopards is not alarming, the small size of many Persian leopard populations and their increasing habitat fragmentation make them potentially prone to genetic drift, inbreeding and reduced gene flow that can decrease their long-term survival (Luo et al., 2004; Uphyrkina et al., 2002; Wultsch et al., 2016). Therefore, further studies are encouraged to apply more genetic markers to reveal fine-scale population structure and variability, particularly along Zagros mountains where the species persistence is highly conservation-dependent.

7.6. References

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Table 7.S1. Reference NADH-5 sequences from NCBI GenBank.

Sequence Name	**Minimum**	**Maximum**	**Length (bp)**
EF551002	12782	13336	555
KP001507	12778	13332	555
KP202265	12486	13040	555
KX655614	11833	12387	555
KJ866876	11828	12382	555
AY035278	62	611	550
AY035276	62	611	550
AY035267	62	611	550
AY035264	62	610	549
AY035274	62	611	550
AY035268	62	611	550
AY035266	62	611	550
AY035262	62	611	550
AY035261	62	611	550
AY035265	62	610	549
AY035263	62	611	550
AY035275	62	611	550
AY035260	62	611	550
GQ244411	62	602	541
JN811046	62	611	550
AY035271	62	611	550
JN811045	62	611	550
JN811043	62	611	550
AY035292	62	611	550
AY035270	62	611	550
AY035273	62	611	550
JN811044	62	611	550
AY035272	62	611	550
KY292255	62	611	550
AY035288	62	611	550
AY035286	62	611	550
KY292277	62	611	550
KY292276	62	611	550

KY292263	62	611	550
KY292262	62	611	550
KY292253	62	611	550
KY292250	62	611	550
KY292237	62	611	550
KY292236	62	611	550
JF720218	62	611	550
AY035291	62	611	550
AY035279	62	611	550
AY035287	62	611	550
KY292259	62	611	550
JF720311	62	611	550
KY292269	62	611	550
KY292266	62	611	550
KY292235	62	611	550
KY292232	62	611	550
KY292226	62	611	550
KY292225	62	611	550
JF720225	62	611	550
JF720224	62	611	550
AY035290	62	611	550
KY292273	62	611	550
JF720264	62	611	550
JF720216	62	611	550
AY035285	62	611	550
KY292228	62	611	550
JF720299	62	607	546
JF720229	62	611	550
KY292245	62	611	550
JF720237	62	611	550
JF720204	62	611	550
JF720203	62	611	550
JF720250	62	610	549
JF720287	62	611	550
AY035281	62	611	550
KY292275	62	611	550

KY292271	62	611	550
KY292267	62	611	550
KY292261	62	611	550
KY292249	62	611	550
KY292246	62	611	550
KY292243	62	611	550
KY292241	62	611	550
KY292239	62	611	550
KY292222	62	611	550
KY292251	62	611	550
HQ185544	60	608	549
HQ185546	60	608	549
HQ185545	60	608	549
HQ185548	60	608	549
HQ185547	60	608	549
HQ185550	60	608	549
HQ185549	60	608	549
JF720243	58	607	550
JF720245	57	606	550
JF720236	55	604	550
JF720214	53	602	550
JF720231	51	600	550
JF720227	46	595	550
JF720208	45	594	550
EF056501	26	575	550
KF768352	2	551	550
KF768354	2	551	550
KF768353	2	551	550

Table 7.S2. Reference CR sequences from NCBI GenBank.

Name	Description	% Identical Sites	% Pairwise Identity	E Value
AF053056	Tiger	100.00%	100.00%	1.53E-31
KR132597	Snow leopard	98.60%	98.60%	4.28E-27
KP202269	Snow leopard	98.60%	98.60%	4.28E-27
JF357971	Indochinese tiger	98.60%	98.60%	4.28E-27
EU872071	Snow leopard	98.60%	98.60%	4.28E-27
DQ151551	Tiger	97.50%	97.50%	3.31E-28
KX655614	Amur leopard	97.40%	97.40%	3.31E-28
KP202265	Leopard	97.40%	97.40%	3.31E-28
KP001507	Leopard	97.40%	97.40%	3.31E-28
KJ866876	Leopard	97.40%	97.40%	3.31E-28
EU872070	Snow leopard	97.40%	97.40%	3.31E-28
EF551004	Snow leopard	97.40%	97.40%	3.31E-28
EF551002	Leopard	97.40%	97.40%	3.31E-28
AY035231	Leopard	97.40%	97.40%	3.31E-28
AY035227	Leopard	97.40%	97.40%	3.31E-28
KR132591	Leopard	96.20%	96.20%	1.54E-26
KR132579	Cheetah	96.20%	96.20%	1.54E-26
KP202271	Cheetah	96.20%	96.20%	1.54E-26
HM748841	Sunda clouded leopard	96.20%	96.20%	1.54E-26
HM748838	Sunda clouded leopard	96.20%	96.20%	1.54E-26
HM748836	Sunda clouded leopard	96.20%	96.20%	1.54E-26
EF440646	Clouded leopard	96.20%	96.20%	1.54E-26
EF440645	Clouded leopard	96.20%	96.20%	1.54E-26
EF434743	Clouded leopard	96.20%	96.20%	1.54E-26
EF434742	Clouded leopard	96.20%	96.20%	1.54E-26
EF434740	Clouded leopard	96.20%	96.20%	1.54E-26
AY463962	Cheetah	96.20%	96.20%	1.54E-26
AY463961	Cheetah	96.20%	96.20%	1.54E-26
AY463960	Cheetah	96.20%	96.20%	1.54E-26
AY463959	Cheetah	96.20%	96.20%	1.54E-26
AY035256	Leopard	96.20%	96.20%	1.54E-26
AY035236	Leopard	96.20%	96.20%	1.54E-26

AH009751	Jaguar	96.20%	96.20%	1.54E-26
AH009740	Jaguar	96.20%	97.10%	3.31E-28
AH009736	Jaguar	96.20%	97.10%	3.31E-28
AF508046	Leopard	96.20%	96.20%	1.54E-26
AF508045	Leopard	96.20%	96.20%	1.54E-26
AF344830	Cheetah	96.20%	96.20%	1.54E-26
KU314499	Domestic cat	95.90%	95.90%	9.26E-24
KU314498	Domestic cat	95.90%	95.90%	9.26E-24
KU314497	Domestic cat	95.90%	95.90%	9.26E-24
KU314496	Domestic cat	95.90%	95.90%	9.26E-24
KU314495	Domestic cat	95.90%	95.90%	9.26E-24
KU314494	Domestic cat	95.90%	95.90%	9.26E-24
JQ245445	Jungle cat	94.90%	94.90%	7.16E-25
KU314491	Domestic cat	94.90%	94.90%	7.16E-25
KU234271	Asiatic lion	94.90%	94.90%	7.16E-25
KR132590	Jaguar	94.90%	94.90%	7.16E-25
KR132589	Lion	94.90%	94.90%	7.16E-25
KP202264	Jaguar	94.90%	94.90%	7.16E-25
KP202262	Lion	94.90%	94.90%	7.16E-25
KP001506	Lion	94.90%	94.90%	7.16E-25
KP001502	Lion	94.90%	94.90%	7.16E-25
KP001501	Lion	94.90%	94.90%	7.16E-25
KP001499	Lion	94.90%	94.90%	7.16E-25
KP001495	Lion	94.90%	94.90%	7.16E-25
KP001494	Lion	94.90%	94.90%	7.16E-25
KP001493	Lion	94.90%	94.90%	7.16E-25
KM236783	Jaguar	94.90%	94.90%	7.16E-25
KF776494	Lion	94.90%	94.90%	7.16E-25
KF483864	Jaguar	94.90%	94.90%	7.16E-25
KC834784	Asiatic lion	94.90%	94.90%	7.16E-25
JQ904290	Asiatic lion	94.90%	94.90%	7.16E-25
HM748840	Sunda clouded leopard	94.90%	94.90%	7.16E-25
HM748839	Sunda clouded leopard	94.90%	94.90%	7.16E-25
EF434744	Jaguar	94.90%	94.90%	7.16E-25

AY035250	Leopard	94.90%	94.90%	7.16E-25
AY035243	Leopard	94.90%	94.90%	7.16E-25
AY035234	Leopard	94.90%	94.90%	7.16E-25
AH009755	Jaguar	94.90%	94.90%	7.16E-25
AH009754	Jaguar	94.90%	96.20%	4.28E-27
AH009753	Jaguar	94.90%	94.90%	7.16E-25
AH009752	Jaguar	94.90%	94.90%	7.16E-25
AH009750	Jaguar	94.90%	94.90%	7.16E-25
AH009749	Jaguar	94.90%	94.90%	7.16E-25
AH009748	Jaguar	94.90%	94.90%	7.16E-25
AH009747	Jaguar	94.90%	94.90%	7.16E-25
AH009746	Jaguar	94.90%	94.90%	7.16E-25
AH009745	Jaguar	94.90%	94.90%	7.16E-25
AH009744	Jaguar	94.90%	94.90%	7.16E-25
AH009742	Jaguar	94.90%	94.90%	7.16E-25
AH009741	Jaguar	94.90%	94.90%	7.16E-25
AH009734	Jaguar	94.90%	94.90%	7.16E-25
AH009731	Jaguar	94.90%	94.90%	7.16E-25
AH009719	Jaguar	94.90%	94.90%	7.16E-25
EF434741	Clouded leopard	93.60%	93.90%	9.26E-24

Chapter 8:

General discussion and conservation implications

General discussion and conservation implications

8.1. Aims and motivations

Humans are responsible for extirpating more large cat species from West Asia and the Caucasus than from any other region in the world (Nowell and Jackson, 1996). The common leopard is the largest widely occurring extant big cat in the region (Sunquist and Sunquist, 2002), and has attracted considerable recent research interest since the early 2000s (Khorozyan, 2003; Kiabi et al., 2002; Lukarevsky et al., 2004; Spalton et al., 2006).

International interest in the region's leopards has been reflected by the publication of three Special Issues in the IUCN/SSC's Cat Specialist Groups' *Cat News*, i.e. "Status and Conservation of the Leopard in the Arabian Peninsula" (2006), "Status and Conservation of the Leopard in the Caucasus" (2007) and "Cats in Iran" (2016). These publications, together with subsequent reports have broadly provided a "documental" baseline (Jacobson et al., 2016) for understanding the leopard's range and distribution. "Fundamental" studies (Jacobson et al., 2016) have been recently conducted in a few reserves (Babrgir et al., 2017; Farhadinia et al., 2014b; Ghoddousi et al., 2017b, 2016, Khorozyan et al., 2015, 2008).

Nevertheless, more applied research articles focused on informing policy, guiding management, or tracking population trends should be expected for subspecies that are at risk of extinction, like *P.p.saxicolor* (Jacobson et al., 2016). Without fundamental science on key biological and ecological questions which provide a foundation for applied researches, the development of sound conservation initiatives is compromised (Balme et al., 2014). In this thesis, my main motivations were to explore key fundamental questions concerning the ecology of Persian leopards and to explore the implications for applied conservation biology. Here, I synthesize the key findings of each chapter and their collective implications for the conservation of Persian leopards.

8.2. Conclusion

8.2.1. Chapter 3: Wolves can suppress goodwill for leopards: Patterns of human-predator coexistence in northeastern Iran

In Chapter 3, I showed that people lost more livestock to diseases than to predators, with leopards responsible for far fewer losses than wolves (as reported by the stockholders). This is associated with a relative greater tolerance of leopards compared with wolves; but the economic loss associated with predator damage (whether caused by wolves or leopards) did appear to influence people's attitude. Therefore, in mosaic landscapes hosting a guild of large carnivores, protecting a single species may depend on the interaction of people with other predators and an understanding of how attitudes to different species interact in human communities may help conservation planning. Measures to reduce conflict not only with the threatened species, i.e. the Persian leopard, but also with the more resilient carnivore, i.e. the wolf are likely to benefit leopard conservation.

8.2.2. Chapter 4: Anchoring and adjusting amidst humans: ranging behaviour of Persian leopards along Iran-Turkmenistan borderland

Leopards in Tandoureh occupied the largest recorded home ranges from Asian landscapes (Karanth and Sunquist, 2000; Odden and Wegge, 2005; Simcharoen et al., 2008), with the exception of an adult male tracked in an arid montane habitat in central Iran (670 km2; Hunter, 2011). The home ranges of predators scale with body mass (Kelt and Van Vuren, 2001) and habitat productivity, which affects prey biomass (Nilsen et al., 2005). The large body masses of Persian leopards (Farhadinia et al., 2014a) and the low primary productivity of the landscapes (e.g. annual rainfall 250-300 mm in northeastern Iran) are likely to be two key determinants of their home range sizes.

Excursions into multiuse landscapes occurred on 17.9% of all leopard tracking days with very wide variation among individuals. Substantial home range overlaps were seen between conspecifics, considerably larger than reported in previous studies on leopards (Bailey, 1993; Fattebert et al., 2016; Marker and Dickman, 2005; Mizutani and Jewell, 1998; Odden and Wegge, 2005). Nonetheless, predation occurred mostly in parts of the home range used exclusively by each leopard, i.e. home range core area. I suggest that this behavioural pattern observed in Tandoureh is attributable to the topographic features of this rugged landscape that can facilitate co-existence of multiple individuals due to restricted detectability for leopards. Amongst home ranges with high degrees of spatial overlap, exclusive hunting areas can facilitate coexistence of multiple individuals.

8.2.3. Chapter 5: Persian leopard predation patterns and kill rates in the Iran-Turkmenistan borderland

I observed a kill rate higher than previously published estimates for leopards. The higher consumption rate can be explained by higher mass-related energetic requirements (Carbone et al., 1999) of Persian leopards as one of the largest subspecies (Stein and Hayssen, 2013). The application of GPS clusters improved the estimation compared with previous techniques, such as spoor tracking, direct sighting and VHF telemetry (Bothma and le Riche, 1982; Martins et al., 2011; Odden and Wegge, 2009). Similarly, higher kill rates were reported for other big cats when GPS clusters were used comparied to previous techniques (Knopff et al., 2010; Miller et al., 2013).

Estimates of kill rate varied almost 2.5-fold among individual leopards. They also showed age-dependent selectivity for certain prey age/sex groups. Prey body mass, a key predictor of prey selection among leopards, explains this selective foraging in different ages. My findings revealed selective hunting of adult male (> 4 years) urial and bezoar goats. Anti-predator vigilance, which is more common in females and kids among mountainous bovids (Cransac et al., 1998; Grignolio et al., 2007), is also a possible explanation.

Wild ungulates dominated leopard diet, as also observed in previous studies based on fecal analysis in northeastern Iran, where 80-95% of the consumed biomass was estimated to be from this source (Farhadinia et al., 2014b; Ghoddousi et al., 2016; Sharbafi et al., 2016; Taghdisi et al., 2013). A single individual (M1) relied mainly on domestic stock, while others predominantly predated on wild ungulates. The longest detected inter-kill intervals were during spring, coinciding with the ungulate birth pulse in Tandoureh. It suggests that leopards shift their spring predation to neonates to some degree. These are rapidly consumed and so do not result in clusters of fixes.

8.2.4. Chapter 6: A paradox of local abundance amidst regional rarity: the value of montane refugia for Persian leopard conservation

I documented the largest number of leopards detected at any location surveyed to date, as well as the highest densities of leopards in the Middle East and the Caucasus. Leopard density estimates in northeastern Iran far exceeded estimates made elsewhere in the Middle East and Caucasus (Hamidi et al., 2014; 0.34 to 2.63 individuals/100 km2; Khorozyan et al., 2008). My estimates were also higher than the majority of published leopard densities across the continental Asian range, with the exception of a few leopard populations in Indian reserves (Goldberg et al., 2015; Kalle et al., 2011). The number of leopards detected in Tandoureh (30 adult leopards) was the largest recorded by the use of camera traps, larger than 72 previous population surveys across the species' global range (see Chapter 6 for available estimates)

The water-based sampling method yielded smaller density estimates but with higher precision than the trail-based method. There are likely to be two underlying reasons. First, the higher number of trail stations compared to water-based cameras (55 vs. 25) and the spatial configuration of trail cameras which provided more coverage (72.3% vs 38.2% of sampling girds) resulted in more individuals being detected along the trails than at water resources (24 vs. 20). Second, cameras at water resources achieved a higher recapture rate (7.9±1.5 vs. 3.4±0.7 captures per individual) and a lower number of individuals with single detections (4 vs. 9 individuals).

8.2.5. Chapter 7: Genetic variation and population structuring of Persian leopards in Iran

I found low haplotype diversity and moderately high nuclear heterozygosity in Iran's leopards. None of the two types of genetic markers showed any evidence of population partitioning, suggesting that Iran's leopards form a single gene pool. Isolation by distance was not evident, showing the high dispersal capacity of leopards (i.e. making isolation less likely).

The used panel of highly polymorphic loci has clarified fine-scale genetic partitioning among Indian leopards (Dutta et al., 2012). As such, my estimate of allelic richness per locus was 2.5 times higher than in a previous analysis with 25 loci on Persian leopards (Uphyrkina et al., 2001). A larger sample size might show fine-scale spatial partitioning with the current panel of loci; however, the subspecies' rarity did not allow me to obtain more samples. Equally important, my mitochondrial data support nuclear findings on lack of spatial subdivision.

Leopards show high mitochondrial and nuclear genomic variability across most of their global range (Anco et al., 2017; Ropiquet et al., 2015; Uphyrkina et al., 2001). Therefore, low haplotype diversity and high nuclear heterozygosity in Persian leopards must be interpreted in the spatial context of Iran, and west Asia. From a demographic perspective, it might be a legacy of bottleneck events in recent or historic times (Ellegren and Galtier, 2016). Nonetheless, no contemporary demographic bottleneck is known from Iran, with a population probably between 550 and 850 individuals and with a wide distribution by the end of the 1900s (Kiabi et al., 2002). Furthermore, leopards show moderate mitogenomic variation, even in areas with substantial population collapse (Ario et al., 2008; Wilting et al., 2016). Alternatively, Persian leopards may have undergone an evolutionarily selective pressure in their mitochondria to adapt to severe oxidative stress and higher metabolic rates required by their cold environments.

8.3. Synthesis and management implications

8.3.1. Living with leopards in a crowded landscape

Chapters 3, 4 and 5 in this thesis provide detailed insight into various aspects of coexistence patterns between leopards and humans in northeastern Iran. Stock raiding by leopards occurs widely in this region (Chapter 3). Although most collared leopard individuals had access to domestic stock when making periodic excursions in human-dominated landscapes outside the national park (Chapter 4), only one leopard (the old male M1) was engaged in severe conflict with communities (Chapter 4 & 5). Therefore, given the presence of staple prey in a livestock free area such as in my study areas, stock raiding is likely to be confined to specific individuals, such as old leopards. In general, problem individuals can be responsible for a disproportionate impact on human interests, such as health, culture, well-being, and economics (Swan et al., 2017).

Prior to making any decision on how to manage the problem-causing individuals, other factors must be considered. For example, M1 (an old male) was severely engaged in stock raiding around Tandoureh. Simultaneously, another old male leopard was involved in a serious conflict around a village named Tazeh Ghaleh, approximately 140 km from our study site, ultimately resulting in its capture and removal (Farhadinia et al., 2015c). Both these leopards caused significant losses for locals (M1 in Tandoureh: 13 dogs + 4 domestic sheep in 11 villages vs. Tazeh Ghaleh: 15 dogs + 2 domestic sheep in one village). Local people showed different levels of tolerance in each case. The Tazeh Galeh leopard was regularly seen in one village where all attacks occurred, resulting in pressure on local authorities for its removal (Farhadinia et al., 2015c). By contrast, the M1 leopard attacks were much more spatially dispersed which may have ameliorated the sense of anxiety in local people. This anecdotal comparison limits the conclusions I draw here but it emphasizes the potential usefulness of tailoring a response that focuses on individual problem leopards as for other carnivores (Linnell et al., 1999). In such scenarios, a clear understanding of both the spatial risk of conflict and the degree of local peoples' grievance would be useful before recommending any management action.

When stock raiding is driven by the behaviour of one individual, as opposed to a behaviour widely occurring across a felid population (Athreya et al., 2016; Babrgir et al., 2017; Lamichhane et al., 2017; Shehzad et al., 2015), selective management to target specific problem individuals can be part of the solution (Swan et al., 2017). Although translocating problem animals has sometimes been successful in the case of some big cats, such as Amur tiger *P. tigris* (Goodrich and Miquelle, 2005), its effectiveness specifically with regard to leopards is ambiguous (Athreya et al., 2011; Linnell et al., 1999; Odden et al., 2014; Weilenmann et al., 2011; Weise et al., 2015). As such, any attempt should be conducted under validated guidelines (Weise et al., 2015), particularly by carefully considering the suitability of recipient locations (Odden et al., 2014; Weilenmann et al., 2011; Weise et al., 2015). The lack of evident spatial population subdivision in Iran's leopards, as revealed by the genetic analysis (Chapter 7) rules out the possibility of genetic admixture following translocating within the region (Ropiquet et al., 2015). Post-release monitoring by fitting leopard individuals with GPS telemetry equipment will be crucial to evaluate the efficiency of such management options.

8.3.2. Functional consequences of leopard predation on ungulate recovery

As a top-down regulatory process, predation influences prey population growth (Gervasi et al., 2011; Wegge et al., 2009) through an additive or compensatory effect (Sandercock et al., 2011). Wild ungulates are also widely targeted by human hunters (Ghoddousi et al., 2017b; Krofel et al., 2014). Although the effect of rampant poaching on multiple ungulates in Iran has been described (Ghoddousi et al., 2017a), the importance of top-down regulation by predators is rarely considered. Thus, understanding how ungulate populations are shaped by different mortality causes is vital for the recovery and conservation of prey, and consequently their predators.

High population density (Chapter 6) and kill requirements (Chapter 5) of leopards highlight the significant role they can play in regulating ungulate populations. In two study sites, all local experts estimated a lower abundance of leopards (as low as 50%) than the number detected by camera traps, when interviewed before the study (Unpublished data). This suggests that the local conservation managers' awareness of the leopard population is imperfect, and consequently that they may underestimate the effect of predation on protected ungulates. If human harvest and predation cause an additive effect on ungulate mortality up to a critical threshold (annual removal levels < 15 %; Wegge et al. 2009), the population may not be able maintain an increasing growth rate. Even when the overall predation rate of a wild ungulate population seems to be close to its balancing recruitment rate, spatio-temporal prey selectivity on certain age/sex classes can also produce different demographic effects (Gervasi et al., 2011; Lovari et al., 2009). Conservation bodies are encouraged to consider the interaction between harvest and predation on ungulate functional response when formulating management plans for rare species, particularly when managing trophy hunting or anti-poaching measures.

8.3.3. Land sharing and land sparing: a combined approach

The highlands of west and central Asia host a low density of wild ungulates, predominantly spatially confined to protected areas, whereas high numbers of domestic animals dominate montane pastures (Baskin and Danell, 2003; Mallon and Zhigang, 2009). These crowded highland pastures with high spatiotemporal variability in resources for animals create formidable challenges for conservation managers attempting to reduce conflict and foster coexistence between humans and top predators. Large cats living in these mountains, already endangered due to habitat loss and persecution (Jacobson et al., 2016; McCarthy et al., 2017), persist at relatively low density (usually <3 individuals/100 km^2; Hamidi et al. 2014; Alexander et al. 2015) compared to large cats in other more productive landscapes (Karanth et al., 2004; Tobler and Powell, 2013). There are also increasing concerns that climate change may have long-term effects on habitat productivity and suitability for larger cats (Ebrahimi et al., 2017; Forrest et al., 2012).

Chapter 8: General discussion and conservation implications

The research findings of this thesis highlight the key role of mountainous ecosystems in supporting a high density of rare apex predators, such as leopards. Well-established mountainous national parks, exclusive of human activities, even comparable to the size of a home range of a single adult leopard, can harbor a high density of the predator (Chapter 6), with the majority of the ranging time spent within the national park boundaries (Chapter 4), preying mostly on wild ungulates (Chapter 5), resulting in negligible conflict with people living around (Chapter 3).

Land sparing (i.e. separation) through establishing national parks ideally with a minimal level of direct human activities as montane refugia is a key achievable solution to help safeguard biodiversity in mountainous areas. In the absence of properly managed protected areas, big cats can experience high rates of human-induced mortality in multi-use lands (Sharma et al., 2014; Swanepoel et al., 2015; Williams et al., 2017).

Obviously, land sharing (coexistence) is also essential, due to the extensive spatial requirements, high energy needs and hierarchical social interaction of big cats which force them to roam beyond reserve boundaries (Johansson et al., 2016; Chapter 4; Macdonald et al., 2010). Arguably, this approach has been efficient to restore large carnivores in many parts of Europe (Chapron et al., 2014; López-Bao et al., 2015), but not in North America (Gompper et al., 2015). Not surprisingly, experiences of land management based only on land sharing are so far worrying in West Asia. Leopards cause severe conflict with communities whenever they have to persist in any West Asian human-dominated area (Babrgir et al., 2017; Dar et al., 2009; Kabir et al., 2013; Shehzad et al., 2015) in contrast to the region’s well-established national parks (Chapter 5; Farhadinia et al., 2014b; Ghoddousi et al., 2016; Sharbafi et al., 2016). Therefore, it is unlikely that land sharing alone can secure the viability of leopard populations, and perhaps of other large predators in Asian mountains.

Thus, in rugged Asian landscapes, a combination of land sparing and land sharing strategies at multiple spatiotemporal scales has the potential to ensure the viability of leopards and other big cats. Properly-managed conservation areas (spared lands) are of paramount importance for “anchoring” high densities of large carnivores, insofar as poaching of carnivores and their prey species is under control. Nonetheless, their use of space outside conservation areas needs to be managed through promoting the existence of carnivores in human-dominated landscapes (land sharing), particularly with minimized levels of conflict with stock breeders.

8.3.4. The importance of mountains for conserving large cats

Mountain ecosystems occupy 25% of the global land surface area (Körner, 2007) and their functional integrity supports a wide range of ecosystem services and biodiversity (Körner, 2004). Mountain regions continue to harbor large numbers of species due to lower levels of human and natural disturbances and more diverse habitats; so they host many biodiversity hotspot ecoregions (Tang et al., 2006). Climate change has significant impacts on species’ distributions and diversity in mountainous areas (Thuiller et al., 2005), forcing many mammalian species to move toward higher elevations and latitudes (Hickling et al., 2006; Levinsky et al., 2007; Luo et al., 2015; Marino et al., 2011). Asian mountains can serve as climate refugia for big cats, despite the fact that only one third of their current extant range is expected to remain as suitable habitat in the future (Persian leopard; Ebrahimi et al., 2017; snow leopard; Li et al., 2016), albeit perceived to be large enough to support viable populations (Forrest et al., 2012; Li et al., 2016).

At the same time, land and water are critical for rural livelihoods and the financial security of communities living in montane landscape (Niroula and Thapa, 2005; Oweis and Hachum, 2006). They are both substantially affected by increasing aridity, threatened food security and human health (Lioubimtseva and Henebry, 2009). Nonetheless, intensified human uses are expected in mountains due to their economic role, associated with more landscape degradation, particularly under climate change (Nogués-Bravo et al., 2007).

Large and intact wilderness inhabited by a sparse human population are correctly prioritized for ecosystem conservation (Mittermeier et al., 2003), with emphasis on larger and better-connected protected areas, particularly for mega-fauna such as leopards (Di Minin et al., 2013). Nonetheless, I argue that conservation policy should not underestimate the value of small mountainous reserves with minimum human disturbance for maintaining biodiversity and ecosystem services. As land use change is the main driving factor for range losses in threatened mammalian carnivores (Di Minin et al., 2016), conservation should clearly be proactive wherever possible. My findings underscore the critical role of small rugged national parks in harboring high densities of breeding populations of wide-ranging apex predators. As such, developing a network of national parks and nature reserves across the Asian mountains should be a clear conservation target in order to secure a high density of breeding nuclei of large cats. Obviously, larger intact areas must generally be sought, but it does not imply that even small areas of rugged terrain should be neglected, when available resources allow bolstering of protection measures.

8.3.5. The future of Persian leopards: northeastern Iran and beyond

Eight Asian subspecies of leopards have experienced between 83 and 87% range loss (Jacobson et al., 2016), and often now occur at perilously low densities (Chapter 6). The endangered Persian leopard is currently perceived to occur across only 16% of its historic range, from Afghanistan through Iran to the Caucasus (Jacobson et al., 2016). Several of the last remnant populations of leopards occur along international borders, such as Iraq, Turkey and Iran (Avgan et al., 2016), the Lesser Caucasus (Askerov et al., 2015; Farhadinia et al., 2015a), and the Kopet

Dag Mountains along the Iran-Turkmenistan borderland (Atamuradov et al., 1999; Farhadinia et al., 2017; Kaczensky and Linnell, 2015). Sadly, some of these areas are badly affected by military conflicts (Avgan et al., 2016; Hanson et al., 2009), and so are not currently accessible for conservation investment. Fragmented landscapes, either mediated by natural causes (e.g. open desert) or anthropogenic factors (e.g. roads, human settlements), have shaped the leopard range in west Asia (Ebrahimi et al., 2017; Gavashelishvili and Lukarevskiy, 2008; Zimmermann et al., 2007), with uncertain connectivity (Farhadinia et al., 2015a). As a result, the long-term viability of some areas depends on immigration from nearby sources which can be through international borders (Askerov et al., 2015; Avgan et al., 2016; Farhadinia et al., 2015a; Khorozyan and Abramov, 2007). Two out of the six collared leopards wandered into Turkmenistan, providing the first evidence of transboundary movement of West Asia’s leopards, underlying the importance of international partnership for protecting shared populations.

Surveys from some of the protected areas of this region have reported densities at the lowest known extreme for the species, fewer than 0.5 individuals/100 km^2 (Khorozyan et al., 2008; Spalton et al., 2006). Well-established reserves in Iran are even known to host low densities of leopards, i.e. < 3 individuals/100 km^2 (Ghoddousi et al., 2010; Hamidi et al., 2014), compared to other subspecies (Chapter 6). Importantly, the few individuals observed in protected areas frequently show no evidence of breeding (Askerov et al., 2015; Avgan et al., 2016; Khorozyan et al., 2014, 2008). Low density and apparent lack of breeding had created general pessimism for leopard survival in the region. My observations show, however, that high population density of leopards is achievable in the region (Chapter 6), provided that prey depletion (bottom-up regulation) and leopard poaching (top-down regulation) are controlled.

In comparison to other parts of West Asia and the Caucasus, leopards in my study areas have higher population size and density (Askerov et al., 2015; Ghoddousi et al., 2010; Hamidi et al., 2014; Khorozyan et al., 2008), low level of conflict, and fairly high public tolerance at the local level (Babrgir et al., 2017; Khorozyan et al., 2015) and infrequent occurrence of deliberate killing of leopards by humans (Sanei et al., 2012). Importantly, most of the suitable leopard habitats in northeastern Iran will sustain their suitability based on different climate change projections (Ebrahimi et al., 2017). Therefore, the Kopet Dag Ecoregion is a significant conservation unit to ensure the long-term viability of Persian leopards. Given moderately high genetic variation and lack of evident population partitioning (Chapter 7), my thesis raises new hopes for leopard viability as well as highlighting the region's potential capacity to restore the historic range of Persian leopards, through dispersal or translocation in Iran and beyond.

8.4. Further research directions

Managing conservation-dependent top predators, such as leopards requires accurate and precise estimates of population size through time and across space. Most studies so far have used camera traps for one-off 'closed' population estimates from single surveys, providing a 'snap shot' of the population status (see chapter 6 for a detailed review on leopard estimates). A few long-term studies have estimated life history parameters and assessed population dynamics for big cats, such as tigers (Karanth et al., 2006), snow leopards (Sharma et al., 2014) and jaguars (Harmsen et al., 2017) as well as leopards (Balme et al., 2009; Rosenblatt et al., 2016). Except for a single Indian study (Harihar et al., 2011), there are no data on life history parameters and long-term trend in abundance for leopard populations in Asia, which mostly occur at alarmingly low density. I recommend the importance of northeastern Iran's national parks, particularly

Tandoureh to be subject for a long-term monitoring program, to inform both leopard population biology and ongoing conservation efforts.

Camera trap studies have been widely used in West Asian and the Caucasian landscapes. They have been used mostly for the purpose of detecting rare species (Abdoli et al., 2008; Avgan et al., 2012; Farhadinia et al., 2009; Ghadirian and Ghasemi, 2012), for population assessment (Askerov et al., 2015; Ghoddousi et al., 2010; Hamidi et al., 2014; Khorozyan et al., 2008; Spalton et al., 2006) or for studying the ranging patterns of big cats (Farhadinia et al., 2016, 2013). Researchers have rarely addressed questions regarding ecosystem function, community structure or biodiversity monitoring (Khorozyan et al., 2014; Rich et al., 2017), resulting in knowledge gaps. This thesis has generated a large amount of photographic data from multiple sites in northeastern Iran. Future studies can combine data sets from multiple studies in order to answer questions more relevant to medium-size mammalian communities in northeastern Iran or to monitor biodiversity indices over time and/or space.

My study provides precise information on kill rate and ranging patterns of Persian leopards in rugged mountains. Obviously, our conclusions are restricted to the sampled cohort of leopards, i.e. adult males. Leopard individual traits, such as age and sex (Bailey, 1993; Balme et al., 2010; Martins et al., 2011; Mizutani and Jewell, 1998) as well as their reproductive status (Rozhnov et al., 2015) are likely to cause differences in predation and movement patterns. Therefore, future studies are encouraged to fill the gap using GPS collaring studies of adult females of different breeding status to quantify sex-specific ranging and foraging behaviours.

Equally important, my thesis research was conducted in a mountainous national park where people are excluded. In order to account for land use regime as a main driving factor for range loss, it is crucial to explore ranging and foraging patterns in leopard behaviour in human-altered landscapes, where the predator inevitably has to share its resources with human and moderate to severe levels of conflict is expected, again by means of GPS telemetry.

There is an ongoing debate on the taxonomy of leopards in West Asia and the Caucasus (Farhadinia et al., 2015b; Khorozyan et al., 2006; Kitchener et al., 2017; Rozhnov et al., 2011; Uphyrkina et al., 2001) and the subspecies nomenclature (Khorozyan et al., 2006; Kitchener et al., 2017). The current study, along with previous molecular investigations (Farhadinia et al., 2015b; Rozhnov et al., 2011) have provided reliable information on phylogeny, taxonomy and genetic variability of leopards in in west Asia and the Caucasus, concluding that the region's leopards possess a monophyletic clade, regardless of its nomenclature. Nonetheless, there are two gaps in knowledge that can improve the conservation needs of leopards in the region:

First, the fragile status of small populationa of leopards in many range countries, particularly the Caucasus, has encouraged governments to develop reintroduction programs (Breitenmoser et al., 2014, 2010; Rozhnov et al., 2011). Originally captured from Iran, Afghanistan and Azerbaijan (Shoemaker, 1985; Uphyrkina et al., 2001), Persian leopards managed by zoos associated with the European Endangered Species Programme (EEP) are candidates for founding these breeding populations. Khorozyan et al. (2006) raised a concern about possible uncertainty of the systematics and geographic origin of the leopards kept in EEP facilities. Then, mtDNA analysis showed that none of captive-born leopards formerly analyzed by Uphyrkina et al. (2001) shared any of haplotypes found in wild-born Persian leopard individuals caught in either Iran or Caucasian countries (Farhadinia et al., 2015b; Rozhnov et al., 2011).

Therefore, I recommend launching a comprehensive genetic study to understand the origin of EEP-managed Persian leopards across Europe using the current study' reference data. Any translocation or breeding program should consider the genetic data beforehand, particularly for those countries where Persian leopards persist. Mitochondrial NADH-5 marker is recommended as a good candidate for this analysis due to its hyper variability in leopards (Anco et al., 2017; Uphyrkina et al., 2001).

Second, there is an increasing interest in exploring the relation between genetic variability and the quality of immunological health in leopards (Castro-Prieto et al., 2011; Parmar et al., 2017). The major histocompatibility complex (MHC) diversity is often linked with the quality of immunological health; the results obtained from a MHC genetic study can increase our understanding about disease predisposition among leopards (Parmar et al., 2017).

Last but not least, I am optimistic that the fundamental topics addressed in this thesis will motivate other biologists to repeat them in other types of landscapes, and to encourage conservation biologists to conduct more applied research on leopards in Iran and beyond.

Chapter 8: General discussion and conservation implications

Life is at risk in the Middle East, due to consecutive catastrophes, from political tensions and military conflicts to severe droughts. Obviously, they do not only threaten humans, but the entirety of biodiversity. There is increasing public pessimism concerning the region and many species are widely perceived to face a gloomy future. I would like to share an optimistic message that there are areas in this region that can motivate conservation, given basic protection is granted and sound science is in action to adapt management efforts.

8.5. References

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Appendix 1. Other publications & activities from the DPhil period

Peer-reviewed papers:

Farhadinia M.S., Hunter L. T. B., Jourabchian A. R., Hosseini-Zavarei F., Akbari H., Ziaie H., Schaller G. B., Jowkar H. 2017. The critically endangered Asiatic cheetah *Acinonyx jubatus venaticus* in Iran: a review of recent distribution, and conservation status. *Biodiversity and Conservation*. 26(5), 1027-1046.

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Book/book chapter

Farhadinia M.S., Moqanaki EM, Shirkhorshidi M, Ekrami B. In press. A Manual on Human-Large Carnivore Conflict Management. Iran Department of Environment.

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Invited lectures

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