Counting bears in the Iranian Caucasus: Remarkable mismatch between scientifically-sound population estimates and perceptions

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Abstract
Lack of reliable data or non-scientific incentives for biased approaches make managers to exclusively rely on experiential knowledge, opinions or perceptions of the status of species, usually derived from personnel belonging to natural resource management agencies. The reliability of this source of information to contribute to the decision-making processes remains doubtful, and largely untested.
We approached this challenging question, common for wildlife monitoring programs in developing countries, using a population of Asian brown bears (*Ursus arctos*) in the Iranian Caucasus as case study. We conducted a noninvasive, genetic, spatial capture-recapture (SCR) study to estimate bear density across an 800-km² core protected area, and compared our estimates of bear abundance with local rangers’ perceptions collated through interview surveys. The estimated average bear density of 4.88 bears/100 km² fell within the range of European bear populations with, reportedly, favorable conservation status. However, the perceived abundance of bears by local rangers was about four times higher than our SCR estimate of 40 bears (2.5-97.5% Bayesian Credible Intervals = 27-70). The vast majority of threatened terrestrial megafauna persist in developing countries, where collection and analyses of demographic data remain challenging. Delayed conservation responses because of erroneous or biased knowledge of population status of such imperiled species may have serious consequences. Our findings offer a reliable baseline for delineating an evidence-based conservation policy for brown bears in Iran, and the Caucasus Ecoregion as a whole.

Keywords: bear abundance, spatial capture-recapture, noninvasive genetic sampling, perceptions, guesstimates, evidence-based conservation
1. Introduction

Reliable information on the status of threatened wildlife populations is essential to inform decision-making processes, assess the degree of compliance with planned conservation goals, or avoid undesirable outcomes from the implementation of interventions (Nichols and Williams, 2006; McCarthy and Possingham, 2007; Jones et al., 2013). Lack of accurate estimates of demographic parameters such as density and abundance, or worse, use of biased information in decision-making may mislead the prioritization of conservation actions (Katzner et al., 2011; Gopalaswamy et al., 2015). Understanding potential confounding factors influencing conservation practitioners and wildlife managers’ judgments about imperiled species, such as those related with the status of populations or the expected impact of interventions, is required to improve current management and conservation practices (Popescu et al., 2016; Heeren et al., 2017).

Insufficient financial resources and logistical constraints imposed by harsh climates or inaccessibility, often prevent conducting reliable population estimates, particularly in developing countries (Danielsen et al., 2009; Karamanlidis et al., 2015). Under this situation, wildlife managers may base their decisions on lower-cost, proxy-based, approaches, usually derived from experiential knowledge, opinions or perceptions of the status of target species (Sutherland et al., 2004; Fazey et al., 2006; Jones et al., 2013; Bennett, 2016). Several studies have pointed out the utility of employing trained local people in monitoring and evaluation of conservation programs (e.g., Steinmetz et al., 2006; Danielsen et al., 2009; Kindberg et al., 2009). However, the reliability of this source of information to form the solid basis for evidence-based practices remains doubtful (Sutherland et al., 2004; Adams and Sandbrook, 2013). Lack of calibration and validation of population estimates may result in wrong decisions and inappropriate allocations of limited resources (Katzner et al., 2011; Gopalaswamy et al., 2015).
An important controversy surrounding large carnivore conservation emerges in relation to the accuracy of the available information on the status of these species, particularly when different stakeholder groups proffer disparate information (Kendall et al., 2009; Chapron et al., 2014; Ripple et al., 2016). Contentious debates about the population estimates of the brown bear (*Ursus arctos*) is such an example. Although effective conservation and management of bear populations are closely tied to the availability of robust estimates of demographic parameters, failure to collect reliable data and use of biased approaches dictated by non-scientific incentives (e.g., trophy hunting) pose a central problem in supporting ecologically-meaningful actions (Bischof et al., 2016; Morehouse and Boyce, 2016; Popescu et al., 2016). Further, the conservation status and allocation of monitoring efforts for bear populations are contrasting across the species’ global range. In Asia, hunting pressure to obtain bear body parts and conflict-related persecution, coupled with the anthropogenic habitat loss, have resulted in a drastic decline of bear populations (Nawaz, 2007; Lortkipanidze, 2010; Latham et al., 2012; McLellan et al., 2017). Particularly in southern and western Asia, reliable data on bear status is extremely limited and many bear populations were extirpated well before any information on their status were available (Bellemain et al., 2007; Garshelis and McLellan, 2011; McLellan et al., 2017). In Iran, brown bears often persist in habitat patches within human-dominated landscapes, and anecdotal sources of information suggest that bear populations are under decline (Gutleb and Ziaie, 1999; Yusefi et al., 2015). Nevertheless, scientifically-sound population estimates of Iranian bears is still lacking, and the available data is based on either experiential knowledge (Gutleb and Ziaie, 1999; Gutleb et al., 2002) or opportunistic visual counts (Farhadinia and Valizadegan, 2015; Parchizadeh, 2017).

The Iranian protected areas (see UNEP-WCMC, 2017) are primarily designed for, and much of the conservation efforts by wildlife managers have been centered around, the conservation of wild ungulates; not only in response to widespread poaching (mainly for meat; Ashayeri and Newing, 2012), but also because the abundance of wild ungulates is generally perceived as an indicator of
local managers’ enforcement effectiveness (Tourani et al., 2014). However, lack of information about the abundance and population trends of large carnivores, such as the case of brown bears, preclude a proper evaluation of the conservation status of this key guild for ecosystem functioning and mitigation of conflicts with human to promote coexistence (Chapron et al., 2014; Ripple et al., 2014). Because of the widespread human-bear conflict, mainly related to bear damages to agricultural products and occasional attacks on humans (Gutleb and Ziaie, 1999; Qashqaei et al., 2014; Yusefi et al., 2015), it is important that future conservation plans for bears in Iran be based upon a realistic knowledge of the status of local bear populations.

Globally, expert and non-expert experiential knowledge have been used in large carnivore monitoring programs, particularly in large populations across regional scales (e.g., Steinmetz et al., 2006; Chapron et al., 2014). Using an Iranian brown bear population and local perceptions about its abundance as case study, we therefore asked the general question: how reliable is the experiential knowledge about the status of large carnivore populations for making sound management decisions? To address this issue, we examined the reliability of local perceptions as the only available source of information that is commonly used as decision-making shortcuts (Bennett, 2016; Heeren et al., 2017), to evaluate whether such heuristics can be used to support management decisions for brown bears in Iran. To do this, we conducted a noninvasive, genetic, spatial capture-recapture (SCR) study to estimate bear density across a core protected area, and compared our estimates of bear abundance with local rangers’ guesstimates collated through interview surveys.

SCR models are advantageous over conventional, non-spatial, analytical approaches. SCR models provide spatially-referenced estimates of density and abundance by linking individual encounter history data with space, and predict a latent variable representing the location and number of individuals’ activity centers (Efford, 2004; Royle and Young, 2008; Royle et al., 2014). The collection of activity centers can be thought of as the realization of a statistical point process describing a
biological pattern. At the most commonly used SCR models such as the half-normal encounter model, the probability of encounter depends on the distance between the detector location and the individuals’ activity centers (Royle et al., 2017). Additionally, the SCR framework can support flexible sampling (i.e., trap) arrangements, and incorporate both individual- and station-level covariates (Sollmann et al., 2013; Efford and Fewster, 2013; Royle et al., 2014; Sun et al., 2014). Therefore, the noninvasive, genetic, SCR approach would be ideal for obtaining reliable estimates of density and abundance for small bear populations. We used our results to expand the current knowledge of Asian brown bear populations, and evaluate how the use of unverified perceptions may influence the interpretation of priorities for conservation and management of such imperiled species.

2. Material and methods

2.1. Study area

Arasbaran Biosphere Reserve (ABR) spreads across approximately 807 km² of the Caucasus Ecoregion in northwestern Iran (38° 40’ to 39° 08’ N, 46° 39’ to 47° 02’ E; Fig. 1). ABR is geographically dominated by mountainous and semi-arid steppe landscapes with elevations ranging from 256 to 2,896 m (Sarhangzadeh and Makhdoom, 2002). Subalpine meadows, grasslands and agricultural lands are intermixed with relatively large patches of temperate mixed broad-leaved forests (Fig. 1) typical from the Caucasus-Hyrcanian biome (Sagheb-Talebi et al., 2013). Aras River marks northern boundaries of ABR with Republic of Azerbaijan (Fig. 1), and several smaller streams draining from this transboundary river flow into the study area. Climate is temperate Mediterranean, and annual precipitation and mean annual temperature vary from 316 to 686 mm and from 5 to 14 °C, respectively (Sagheb-Talebi et al., 2013).

ABR shows relatively high levels of anthropogenic disturbance with at least 66 inhabited villages and thousands of nomads occurring inside the reserve, and a human population density of >18.0 people/km² (www.amar.org.ir). Local people are mostly agro-pastoralists who graze the entire ABR,
with the exception of the study area’s ca. 90-km² core zones (Fig. 1) that collectively were upgraded to a legal status of National Park in 2012. Sheep (*Ovis aries*), cattle (*Bos taurus*), and goat (*Capra hircus*) are stocked at remarkable densities within ABR (>104.4 livestock units/km²; Sarhangzadeh and Makhdoum, 2002). Seven permanent and two seasonal ranger stations guarded ABR during this study (Fig. 1). Each ranger station was responsible for a patrol section within ABR. Although exact geographical boundaries did not exist for patrol sections, each section was defined according to geographical features, nearby human population areas, road access, and location of wild goat’s (*Capra aegagrus*) core habitats (Fig. 1).

2.2. Noninvasive genetic sampling

In 2012, we collected bear feces between July 3 and September 17 (10 weeks) in ABR, within the same period of the bear annual cycle (hyperfagia) and just after the peak of infanticides reported in bear populations from similar temperate regions (Steyaert et al., 2012). This design reduced potential violations of population closure assumptions. We divided ABR into eight sampling areas based on the existing patrol sections at the time of this study (Fig. 1). We followed a single-sampling occasion approach (Bellemain et al., 2007; Puechmaille and Petit, 2007; Royle et al., 2014; Bischof et al., 2017), surveying each section once. Sampling was opportunistic in potential bear habitats that were identified via interrogating rangers and local villagers (Moqanaki et al., 2013). Surveys in each section lasted 3-5 days depending to the availability of potential bear habitats, accessibility, and logistical constraints, and all survey routes were recorded with a GPS unit. We did not sample the sections along the Aras River, as they were dominated by large towns and human infrastructures (Fig. 1).
Bear feces were recognized by their shape, size and, often, presence of large volumes of poorly-digested plant materials. For each scat collected, we carefully stored it in a paper envelope, and we recorded the spatial location using a handheld GPS, as well as the approximate age. We never encountered feeding sites or piles of feces, so all the feces encountered were treated as one independent sample. Within 12 hours from collection, approximately 1-2 cm\(^3\) of the outer layer of each fecal sample was individually transferred by flamed razors into 10- or 50-mL plastic capped tube containing 95% ethanol in 1: ≥4 ratios. Samples were kept at ambient temperature in dark (maximum of 4 months) and stored at -80 °C once at the DNA lab.

Because collection of only fresh feces may not meet the required distribution and quantity of fecal-DNA samples given our single-sampling occasion approach, we followed a more optimistic sampling design and all the relatively old to very fresh bear feces that appeared intact were collected, including weathered samples and even feces with negligible levels of insect activity or occurrence of mold. We acknowledged that this sampling approach might decrease the DNA amplification success and probably increase the genotyping error rates (Murphy et al., 2007 and references therein) but, it increased the chance of identifying new bear individuals and the number of recaptures. We performed an experimental test of decomposition rate of fresh feces in our study area (Moqanaki et al., 2013). In the presence of rain, mold and insects, most of the bear feces would disappear or become degraded in ≤5 days at lowland forests, and up to 10 days in drier montane habitats. Based on these preliminary results and the approximate age of the bear feces found, we are confident that our sampling design only included feces within the sampling period.

Detailed description of DNA extraction and screening of samples for their quality with mitochondrial DNA analysis can be found in Supplementary Material and Moqanaki et al. (2013). Samples that were successfully amplified for a 189-basepairs fragment of cytochrome b (cyt\(b\)) to withdraw low quality samples, were then typed for seven dinucleotide microsatellite loci and one sex.
determination locus (details in Table S1, Supplementary Material). Microsatellite primers, PCR protocols, protocols for individual and sex identification, genotyping reliability and reducing genotyping errors are described in Supplementary Material. We estimated the standard genetic diversity parameters for each locus, and deviation from Hardy-Weinberg equilibrium for each locus and across all loci, with GENEPOP V4.0 (Rousset, 2008) using an exact test and a Markov chain method for loci with ≥5 alleles.

2.3. Bear density and abundance estimation

We used an SCR modelling framework (Efford, 2004; Royle and Young, 2008; Gardner et al., 2009), with the approach proposed by Russell et al. (2012) and Royle et al. (2014), to estimate the density and abundance of bears in ABR using the genotyped fecal samples. The model addresses the movement of individuals by assuming that each individual has an activity center and that the probability of capture is a function of the distance from the activity center to a detection location. The SCR model assumes that every individual in the population has its own activity center (and stationary during sampling, but see Royle et al., 2016), and that all activity centers are distributed uniformly across the study area. The latent variable in SCR is the location and number of individual’s activity centers (Royle et al., 2017), which are estimated across the state-space.

Considering the available information on year-round home range sizes (95-100% Minimum Convex Polygon estimates) of adult brown bears, including dispersing individuals, in the Caucasus and Southeastern Europe (males: mean= 537.3 km², 10%-90% CIs= 174.0-972.4; females: mean= 79.2 km², 10%-90% CIs= 19.5-160.3; Table S2 in Supplementary Material), we created a cell grid layer of 4.5 x 4.5 km (20.25 km²) over the study area. We used the center of sampling grid cells as conceptual “traps” or detectors following Russell et al. (2012). This cell size accounted for 25% of the estimated average home range size of adult female bears, and was selected based on previous suggestions on maximum space between detectors, that is around 2 times the scale parameter sigma (σ, the
parameter determining the decline of detection frequency of individuals in detectors with increasing distance from their activity centers; Sollmann et al., 2013; Sun et al., 2014). Such small cell size avoided an excessive loss of resolution in $\sigma$ (see below). All the genotyped fecal samples were assigned to the centroids of detectors (Gardner et al., 2009; Russell et al., 2012). Because each centroid was considered as a detector, $\sigma$ is estimated from the distribution of distances of the same individuals in different centroids (Russell et al, 2012; Royle et al., 2014).

We assumed that every individual $i$ in the population had its own activity center $s_i$, and that all these activity centers would be distributed randomly across the study area. The position of centroids $j$ was $x_j$ and the encounter histories was $y$, which in this case is a bi-dimensional matrix "$i \times j$", because there was only one sampling occasion. The number of times that an individual $i$ was located in a centroid $j$ is Poisson-distributed (i.e., multiple captures can occur in the same centroid), with mean $\lambda_{ij}$:

$$ y \sim \text{Poisson}(\lambda_{ij}) $$

Occasions (i.e., repeated opportunities for observation) in both spatial and non-spatial hierarchical models can be accomplished through structuring in both space and time (i.e., visiting one site multiple times or visiting multiple sites, survey routes, or points within a spatial unit). Count-based observation models, such as the Poisson-distributed model, allow effective parameter estimation using multiple detections of the same individual at the same detector using only a single survey, although it is constrained in the use of temporal or behavioral covariates (Royle et al., 2014).

Detection probability is a decreasing function of distance between the activity center of the individual and the location of a detector. The expected relationship between the distance from activity center to detector location is negative and nonlinear. The link function between the location
of detectors and the activity centers for individuals follows a half-normal distribution (Royle et al., 2014):

\[
\lambda_{ij} = \lambda_0 \times e^{\left(\frac{-1}{2\sigma^2} \times d_{ij}^2\right)}
\]

, where \(d_{ij}\) is the distance between the activity center for each individual \(s_i\) and the centroid of the detector \(x_j\), and \(\lambda_0\) is the baseline encounter probability (i.e., the encounter probability at the activity center), which depends in our model on sampling effort in each grid cell:

\[
\log(\lambda_0[j]) = \alpha_0 + \alpha_2 \times L[j]
\]

, where \(L[j]\) is length of survey (km) in each cell corresponding to the centroid \(x_j\). Therefore, we used sampling effort as covariate from basal detection rates.

The total number of activity centers (\(N\)) is estimated in the model applying the data augmentation approach (Royle et al., 2014) by adding potential individuals with all zero encounter histories. The state-space (\(S\)) is generated as a rectangle centered on the study area and adding a distance buffer to the grid of centroids. Such distance must be \(>2.5\sigma\) (Royle et al., 2014). In our case, we added a distance buffer of 15 km. Cells beyond a 2.5 \(\sigma\) buffer will have a negligible detection probability and, therefore, density estimates will be equal to the mean density estimate in the state-space (Royle et al., 2014).

We implemented this model in a Bayesian framework using NIMBLE (NIMBLE Development Team, 2015) and R 3.2.4 (R Development Core Team, 2016). We ran 3 chains of the MCMC sampler with 50,000 iterations each, yielding 150,000 total samples from the joint posterior distributions. To check for chain convergence, we calculated the Gelman-Rubin statistic R-hat (Gelman et al., 2013).
Values below 1.1 indicated convergence. Because of the few spatial recaptures (see Results), we used an informative prior for sigma. We considered a conservative value of sigma 0.4, equal to 4 km, (SD = 0.12) based on the published average home range size estimates and variability in home range sizes from neighboring bear populations (Table S2 in Supplementary Material). The informative prior for sigma was calculated as follow:

\[ \hat{\sigma}_{hr} = \sqrt{\frac{A}{\pi}} \frac{1}{q_{2,\alpha}} \]

where \( q_{2,\alpha} \) was the value of a Chi-square with 2 degrees of freedom (\( \alpha = 0.05, q_{2,0.05} = 5.99 \)) and \( A \) (in \( m^2 \)) was the estimated average home range size for adult brown bears, including dispersing individuals, extracted from the literature (313.45 \( km^2 \); calculated by averaging values reported in Table S2, Supplementary Material). We scaled the sigma parameter by 10,000. Non-surveyed grid cells were excluded from the analysis.

We evaluated the goodness of fit of the model by using the Bayesian p-value approach described in Royle et al., (2014; see also Gelman et al., 1996). We tested three fit statistics: i) individual x trap frequencies, which summarizes the data by aggregating individual and detector-specific counts; ii) individual encounter frequencies, which evaluates heterogeneity in encounter frequencies due to space; and ii) detector frequencies, which is based on aggregating over individuals and replicates to form centroid-encounter frequencies.

We used the mode to report the density of bears because of the asymmetry observed in the posterior distribution of this parameter (Royle et al., 2014). The SCR model assumes that individuals are uniformly and independently distributed over the state-space \( S \) (Royle et al., 2014). Therefore, we assumed that bear density was uniform between surveyed and non-surveyed cells (Fig. 1).
2.4. Rangers’ perceptions of bear abundance

We developed a semi-structured questionnaire to evaluate rangers’ perceptions about the abundance of bears within ABR. Rangers were all male, divided into groups of 2-3 persons in each station during two-week shifts. In total, 26 rangers worked in ABR during this study. Prior consent was obtained for all respondents, after the goal of the study was explained and confidentiality assured. Interview surveys were carried out on a one-to-one basis, through face-to-face interviews (n=11) or by phone calls (n=13) in three consecutive days in August 2012. Thus, we avoided that respondents could be influenced by their colleagues through potential discussions about the questionnaire and our goals. All data collection was done by the first author for consistency. Two rangers refused to participate in the survey during the interview period.

Using a topographic map of the patrol sections labeled with local names, we asked each ranger to guesstimate the minimum and maximum number of bears (as the upper and lower bounds) within each patrol section during the study period (Fig. 1), based on his experience and knowledge of ABR bears and the study area. To improve the accuracy of the rangers’ guesstimates, and to reduce the potential overconfidence, interviewees were invited to provide more thoughtful guesstimates by clarifying that: (1) we were interested in each respondent’s personal opinion, thus there were no good or bad answers; and (2) respondents were free to provide guesstimates for only those patrol sections they had worked in (see Results). Lastly, we gathered information on several factors that could influence rangers’ perceptions of bear abundance in ABR: (1) socio-demographics: birthplace, age, education level; and (2) experience-related factors: job status, number of years working as a ranger in ABR, and number of patrol sections worked in ABR.

The qualitative data (birthplace, education level, job status) were scaled and, together with the quantitative data (age and experience-related variables), were entered into a data matrix as
numbers without any transformation. Rangers’ birthplace and job status were coded as binary variables. “Local” ranger (= 1) was inhabitant of, or had spent the majority of his life in, villages in or in vicinity (≤10 km) of ABR, against an “outsider” ranger (= 0). “Full-time” (= 2) and “part-time” (= 1) rangers were identified based on each ranger’s employment status during the interview survey. Full-time contracts were casual rangers who were offered a permanent position and participated routinely in field patrolling. In contrast, part-time employed rangers (agency workers) received lower salaries under short-term contracts, thus they were expected to contribute less frequently in patrolling and anti-poaching activities. The education level was described with a three-grade scale (low-illiterate or primary education = 1; lower, upper or post-secondary education = 2; and high school diploma, pre-university or university degrees = 3).

We pooled all ranger guesstimates and calculated the median values of minimum and maximum bear guesstimates reported per patrol section. By combining the minimum and maximum medians per section we obtained the guesstimate of bear abundance for the entire ABR. Because several rangers provided guesstimates only for a number of patrol sections (see Results), we calculated the combined median of minimum and maximum guesstimates of bear numbers for two groups of rangers separately, namely, “total rangers” (i.e., those rangers that agreed to participate in the study; n = 24) and “volunteered rangers” (i.e., those interviewed rangers who volunteered to provide their perceptions of bear abundance for all the patrol sections; n = 10).

We employed non-parametric tests to investigate whether rangers’ perceptions of bear abundance in ABR were influenced by socio-demographic and experience-related attributes (independent variables). Mann-Whitney U tests were used to test for differences in bear guesstimates between ranger groups (local vs. outsider and full-time vs part-time rangers); whereas Spearman’s correlation tests were used to evaluate the influence of continuous factors, such as age and other experience-related characteristics of interviewed rangers, on rangers’ guesstimates. We did not test the effect
of education levels on rangers’ guesstimates because the number of cases by defined classes was not enough (see Results).

3. Results

3.1. Genotyping success and bear individual identification

We collected 109 bear feces along 206 km of survey routes within ABR (Fig. 1). Two fecal samples were initially discarded because of high prevalence of mold, and bear DNA from the remaining were extracted at least once. Out of 107 samples, 64.5% (n = 69) were successfully amplified for the cyt b fragment, and were used in the microsatellite genotyping. We successfully genotyped 45 samples (65.2% of the screened samples using the cyt b fragment, or 42.1% of the total extracted DNA samples) for eight loci (Tables S1 and S3 in Supplementary Material). Overall, we identified 31 bear individuals in our dataset, with unique multi-locus genotypes detected between 1 and 4 times (mean = 1.4 ± 0.7 SD); i.e., 21 bear individuals were detected once, 8 bears twice, 1 bear three times, and 1 bear four times. We successfully assigned the sex to 30 (96.8%) individuals (19 males and 11 females). Mean distance between the individual bear recaptures (i.e., ≥2 detections) was 1.40 km (± 2.34 SD, range: 0.04 - 7.49 km). Only two (6.5%) genetically-identified bear individuals (two males) were detected in more than one cell.

Descriptive genetic parameters are shown in Table S3 in Supplementary Material. The unbiased Probability of Identity (P_{ID}) and P_{ID} among siblings (P_{SIB}) scores were 1.981 \times 10^{-9} and 0.0008, respectively, showing that we had enough number of markers to reliably differentiate between bear individuals. No significant deviation from Hardy-Weinberg equilibrium was observed (Table S3 in Supplementary Material). The overall multi-locus inbreeding coefficient value (F_{IS}) was 0.074.

3.2. Bear density and abundance estimates
Out of the fifty-five 4.5 x 4.5-km cells in ABR, 43.6% (n = 24) were sampled (Fig. 1). A positive relationship between bear detection probability and length of survey (m) per cell was observed (Fig. S1 in Supplementary Material). Thus, by surveying a minimum length of 15 km per cell we estimated an individual bear detection probability of >0.5 by means of genotyped bear feces (Fig. S1 in Supplementary Material). Our SCR model yielded a density estimate of 4.88 bears/100 km² within ABR (2.5-97.5% Bayesian Credibility Interval [BCI] = 3.38-8.69; Fig. 2, Table 1). Accordingly, the estimated ABR bears abundance was 40 bears (2.5-97.5% BCI = 27-70), taking into account all age classes from cubs of the year after the peak of infanticide. Posterior summaries of model parameter estimates are shown in Table 1. Bayesian p-values showed a good fit for the case of individual x detector encounter frequencies (P = 0.316) and for individual frequencies (P = 0.485), and poor fit (P = 0.0014) for detector frequencies (Fig. 3).

3.3. Rangers’ perceptions of bear abundance

The interviewed rangers (n = 24) aged between 25 and 48 years, with “outsider” rangers (n = 14, 58.3%) slightly outnumbering their “local” colleagues (Table 2). The majority of rangers hold university degrees (Table 2). One-third of rangers described themselves as “agency worker” allocated to seven (87.5%) different patrol sections across ABR. Interviewed rangers had experience of working on average in 3 different patrol sections in ABR (±1.5 SD), and their experience varied between 8 months and 26 years during this study (Table 2). A wide range of perceived bear abundance per patrol section was provided (range: 8 - 21 ranger-perceived minimum and maximum bear guesstimates per patrol section; Fig. 4).
The perceived abundance of bears within ABR by all the interviewed rangers (i.e., “total” rangers in Table 2) was (median) 156 bears ± 7.3 SD. However, out of the 24 rangers that agreed to participate in this study, only 10 (41.67%) volunteered to provide their perceptions on bear abundance in the eight patrol sections in ABR (i.e., “volunteered” rangers in Table 2). Volunteered rangers showed similar socio-demographic and experience-related attributes in comparison to the total rangers (Table 2). The perceived abundance of ABR bears reported by volunteered rangers (median = 146 bears ± 7.1 SD, range: 64-269 bears) was almost four times higher than the noninvasive, genetic, SCR estimate of abundance (mode = 40 bears; 2.5-97.5% BCI = 27-70). These figures were used for testing the effect of the socio-experience variables on rangers’ perceptions of bear abundance within ABR.

We did not observed significant differences in perceived bear abundance between “local” and “outsider” volunteered rangers (Mann-Whitney U test = 10, P = 0.748), or between “full-time” and “part-time” volunteered rangers (Mann-Whitney U test = 9.5, P = 0.909). Additionally, neither the age of respondents (r_s = 0.14, S = 140.71, P = 0.674) nor the experience-related variables of years of work experience as ABR ranger (r_s = -0.24, S = 205.75, P = 0.505) and number of patrol sections that volunteered rangers had worked in (r_s = -0.63, S = 270.06, P = 0.052) significantly influenced their guesstimates of bear abundance.

4. DISCUSSION

Using an SCR modelling approach, we were able to estimate the density and abundance of a small population of brown bears in the Iranian Caucasus with irregular sampling design and low detection rates. Our study provides the first reliable assessment of a brown bear population in Iran, and one of
few that to date have applied SCR models for such a purpose on Asian brown bears (Latham et al., 2012). Considering the posterior estimate for sigma (0.4; 97.5% BCI = 0.33 - 0.53), and the general recommendation that the maximum detector/trap spacing should be around $2\sigma$ (Sollmann et al., 2013; Sun et al., 2014), the grid cell size we chose (4.5 x 4.5 km cells) was adequate for our case study ($\sigma = 0.4$, equal to 4 km). Our SCR model showed a good fit for explaining the individual frequencies by detector and individual heterogeneity. The estimate would be improved in the future with more spatial recaptures (e.g. by increasing the sampling effort) to optimize the sigma calculation.

The conservation community has been criticized because of focusing on rarity and endangerment, overlooking the value of “common” species (e.g., Redford et al., 2013). Yet, the notion behind defining commonness itself might be locally skewed and loosely based on scientifically-sound information, as we illustrated in our case study of ABR bears. Although demographic parameters such as density and abundance, as well as spatial distribution, are commonly used to estimate the relative likelihood of species extinction (see IUCN, 2012), there is no silver-bullet answer from population estimates similar to our study to simply infer about the status of a large carnivore population as “healthy”, “favorable”, “satisfactory”, or “reasonable”. Cautious reference to the available information from the neighboring bear populations can be helpful in clarifying the relative conservation status of ABR bears. Nevertheless, direct comparison of density and abundance estimates between brown bear populations might be misleading because of different sampling and analytical approaches used across studies. For example, total (Ambarli, 2006) or females-with-cubs observation counts (Solberg et al., 2006), integration of GPS-telemetry data with other sources of occurrence such as sign surveys (Jerina et al., 2013; Popescu et al., 2017), and noninvasive DNA sampling of feces (Bellemain et al., 2007) and hair-snagging (Latham et al., 2012) have been used to detect bear individuals at small and large spatial scales for population estimates of brown bears. In addition, non-spatial, effort-corrected observation indices (Kindberg et al., 2009), rarefaction curves...
(Bellemain et al., 2007), conventional capture-recapture designs (Ciucci et al., 2015), and SCR modelling (Karamanlidis et al., 2015; Popescu et al., 2017) have been used to estimate the density and abundance of Eurasian brown bear populations. Our density estimate of 48.8 bears/1000 km$^2$ in ABR was high compared to the bear densities reported from Northern Pakistan (10.7 - 19 bears/1000 km$^2$; Bellemain et al., 2007) and the Greater Caucasus (13 bears/1000 km$^2$; Lortkipanidze, 2010), similar to recovering bear populations in the Italian Alps (20 - 40 bears/1000 km$^2$; Tosi et al., 2015), Central Apennines (39.7 bears/1000 km$^2$, Ciucci et al., 2015) and three Mediterranean populations in Greece (50 - 54 bears/1000 km$^2$; Karamanlidis et al., 2015), but considerably lower than a human food-reliant bear population in northeastern Turkey (110 - 270 bears/1000 km$^2$; Ambarlı, 2006), as well as the Romanian Carpathians (113 - 124 bears/1000 km$^2$; Popescu et al., 2017) and Slovenia (130 bears/1000 km$^2$; Jerina et al., 2013). Further, average allelic richness (8.6), heterozygosity ($H_e = 0.80$), and inbreeding value ($F_{IS} = 0.074$) suggest that the ABR bear population is not at immediate risk of inbreeding depression (Bellemain et al., 2007; Skrbinšek et al., 2012). Thus, one might assume that ABR features a bear population with good conservation status. However, we observed substantial variation in the perceptions of local rangers on bear abundance in ABR, in which most of them proposing ecologically-unrealistic high bear guesstimates (Fig. 4). The majority of respondents were confident in declaring that “an extremely abundant bear population persists in the study area”, with an average perceived bear abundance of between 3.7 (volunteered rangers) and 3.9 times (all the interviewed rangers) higher than the genetic SCR estimate of 40 (2.5-97.5% BCI = 27 - 70) bears.

Evidence-based experiential knowledge, not perceptions or opinions, can sometimes form the best available information to inform decision-making processes (Danielsen et al., 2009; Bennett, 2016). Some have suggested that incorporation of non-expert experiential knowledge into appropriate scientific methodologies is capable of contributing in population assessments of imperiled species (Fazey et al., 2006; Steinmetz et al., 2006; Kindberg et al., 2009). We acknowledge that, in practice, experiential knowledge may support simplified decision making shortcuts, with implications to
address appropriate management and conservation actions (Heeren et al., 2017). However, our results suggest that using experiential knowledge, without being tested properly, may give a skewed portrait of the status of locally perceived “common” species, such as the case of brown bears in ABR.

Besides the local perceptions of ABR bear abundance, at the national level, the Iranian Department of Environment refers to a rough expert-based guesstimate of 150 bears in the management plan for ABR (Shahbazi, 2002), whereas a population of ≤100 bears is suggested for the entire Iranian Caucasus (Gutleb et al., 2002). Disagreement between the scientifically-sound population estimates of brown bears and expert-based guesstimates is reported elsewhere (e.g., Solberg et al., 2006; Kendall et al., 2009; Latham et al., 2012), yet previous studies did not attempt to empirically compare reliable estimates against experiential knowledge and perceptions. We argue that, where either expert or non-expert experiential knowledge is planned to be integrated into wildlife management and monitoring programs, it is crucial to experimentally test the reliability of such sources of information.

Our interview survey provides evidence that by increasing the experience of rangers in terms of number of patrol sections they had directly worked in, they tend to provide more conservative guesstimates of bear abundance. We do not consider that rangers per se have local knowledge of status of ABR bears. However, we hypothesized those “local” rangers who are native to ABR, may have acquired practice-based knowledge of this environment through a long history of exposure to it (Adams and Sandbrook, 2013). Therefore, their perceptions of ABR bear abundance would tend to be closer to our genetic SCR estimate. Rejection of this hypothesis in our case study, however, may be due to the small sample size of rangers interviewed (i.e., only 10 volunteered rangers), and that the relevance and depth of rangers’ knowledge on large carnivore abundance may not correctly reflect their potentials for contributing in local decision-making processes.
Several reasons may be behind the perceptions showed by ABR rangers on the abundance of bears. First, brown bears are large-bodied carnivores that tend to forage on predictable feeding grounds even on highland open terrains (Bellemain et al., 2007; Farhadinia and Valizadegan, 2015; Penteriani et al., 2017), making their observation a common experience for ABR rangers. During our interview survey, we found out that rangers commonly refer to their field observations of multiple bears or females with cubs on such feeding hotspots, and making their guesstimates based on wrongly extrapolating this figure to the entire ABR. It is important to stress that the frequency of observation of bears, or any conventional visual counts, do not necessarily resemble their population density and abundance (Solberg et al., 2006; Kendall et al., 2009; Katzner et al., 2011). Second, the relationship between rangers and ABR residents may also contribute in shaping the perceptions about the abundance of ABR bears and, in turn, the idea of commonness. Although rangers were primarily responsible for law enforcement against poaching, they were also expected to address human-wildlife conflicts in the study area. In ABR, local people show negative attitudes towards brown bears, and frequently complain about the bear damages to their agricultural products or occasional attacks on humans (E. M. Moqanaki, unpubl data). The frequency and intensity of damage claims may have influenced ranger perceptions about the status of ABR bears. Third, our SCR estimate refers only to bears whose home ranges are centered within ABR. The density estimate is obtained for the entire state-space ($S > 2.5\,\sigma$), but the estimate of bear density for ABR only consider those individuals with their activity centers within ABR. Some bears, however, with their activity centers outside ABR, may eventually range within the study area, and vice versa, influencing perceptions on bear abundance (Bischof et al., 2016). Fourth, we cannot exclude overestimation in bear guesstimates by ABR rangers for neighboring patrol sections because the same observed bears may range over several patrol sections. By gaining a better understanding of the study area through working in more patrol sections, rangers may rely less frequently on claims about bear abundance by inexperienced colleagues and local residents, adopting their perceptions of bear status based on their own experience of the commonness of ABR bears.
Large-bodied mammalian carnivores intrinsically attract the largest amount of research and conservation attention from the public and natural resource management agencies (Ripple et al., 2014; 2016). Obtaining high quality individual-based data for monitoring these species is costly, being usually constrained by the time and resources invested (Jones et al., 2013; Bennett, 2016). Delayed conservation responses because of lack of knowledge on population status of imperiled species may even result in local extinctions. One recent example is the extinction of the Javan rhino \(\text{(Rhinoceros sondaicus annamiticus)}\) from Vietnam, which once was perceived to be locally viable, but low political will to take adequate conservation-related actions in time led to its tragic extinction (Brook et al., 2014). Pro-active conservation actions are cost-effective and less management-demanding than considering actions only when target populations are trapped in an extinction vortex (McCarthy and Possingham, 2007; Redford et al., 2013). For ABR bears, basing conservation and management priorities with the current perceptions of the population status can have serious management and conservation consequences. Shortly after our study period, the wildlife authority reported that at least 10 bears had been illegally killed by reserve residents in ABR within one year (Masoud, 2014) suggesting a lack of effective conflict management and law enforcement in the study area. Biased estimates of bear abundance and a false sense of immunity from extinction for a locally-perceived common species result in erroneous assessment of threats to ABR bears by the local wildlife authority. Based on this study, we recommend wildlife managers in Iran and elsewhere to take into account reliable population estimates derived from SCR models, instead of the conventional count-based methods, experiential knowledge or perceptions that are prone to give a biased portrait of the study population (Russell et al., 2012; Bischof et al., 2016; Popescu et al., 2016; Royle et al., 2017). Even thereafter, there is no mechanism to ensure that the information on the status of ABR bears derived from this study would be considered in future conservation plans of ABR. As the vast majority of threatened terrestrial megafauna range are in developing countries (Ripple et al., 2016) with limited capacity of obtaining reliable monitoring data, it is crucial that the
focus of research would be allocated on supporting evidence-based conservation actions, and test
the reliability of current sources of information for the monitoring of populations. However,
availability of population-level monitoring data does not guarantee their incorporation into
conservation actions by the focal administration units. Replacing unverified experiential knowledge
or perceptions with evidence-informed sources of information requires substantial restructuring of
current practices used by conservation practitioners and an efficient communication platform
between scientists and decision-makers (Sutherland et al., 2004; Adams and Sandbrook, 2013).

SUPPLEMENTARY MATERIAL
Supplementary data to this article can be found online at xxx.

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REFERENCES


Masoud, M., 2014. Bears raid villages - 10 bears killed in Arasbaran [in Persian].

Masoud, M., 2014. Bears raid villages - 10 bears killed in Arasbaran [in Persian].


Shahbazi, Y., 2002. Management plan for Arasbaran Protected Area: fish and wildlife studies (Vol. 11). The Iranian Department of Environment, Tehran, Iran, 128 pp. [In Persian].


UNEP-WCMC, 2017. Protected Area Profile for Iran (Islamic Republic Of) from the World Database of Protected Areas, October 2017. Available at: www.protectedplanet.net

Table 1. Posterior summaries of parameter estimate for density estimation of brown bears in Arasbaran Biosphere Reserve (ABR), Iran.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Mode</th>
<th>SD</th>
<th>2.50%</th>
<th>50%</th>
<th>97.50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>alpha0 *</td>
<td>-1.38</td>
<td>-1.32</td>
<td>0.28</td>
<td>-1.95</td>
<td>-1.37</td>
<td>-0.86</td>
</tr>
<tr>
<td>alpha2 *</td>
<td>0.54</td>
<td>0.51</td>
<td>0.16</td>
<td>0.24</td>
<td>0.54</td>
<td>0.85</td>
</tr>
<tr>
<td>sigma</td>
<td>0.41</td>
<td>0.40</td>
<td>0.05</td>
<td>0.33</td>
<td>0.42</td>
<td>0.53</td>
</tr>
<tr>
<td>Psi</td>
<td>0.38</td>
<td>0.34</td>
<td>0.08</td>
<td>0.24</td>
<td>0.38</td>
<td>0.39</td>
</tr>
<tr>
<td>D</td>
<td>5.48</td>
<td>4.88</td>
<td>1.38</td>
<td>3.38</td>
<td>5.28</td>
<td>8.69</td>
</tr>
</tbody>
</table>

* alpha0 and alpha2 are the parameters for $\log(\lambda_0)$ where $\lambda_0$ is the baseline encounter probability; sigma (\(\sigma\)) is the parameter of scale or movement; psi is the data augmentation parameter, and D is the bear density (bear individuals/100 km$^2$). For all parameters, Rhat < 1.1.
Table 2. List of socio-demographic and experience-related variables considered in the analysis of rangers’ perceptions of bear abundance in Arasbaran Biosphere Reserve (ABR), Iran.

<table>
<thead>
<tr>
<th>Rangers ¹</th>
<th>N</th>
<th>Age ± SD</th>
<th>% Birthplace</th>
<th>% Education</th>
<th>% Job</th>
<th>Experience ± SD</th>
<th>Patrol sections ± SD</th>
<th>Guesstimate ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Local</td>
<td>Outsider</td>
<td>Primary</td>
<td>Secondary</td>
<td>University</td>
<td>Casual</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>33.4 ± 5.9</td>
<td>41.7</td>
<td>58.3</td>
<td>20.8</td>
<td>16.7</td>
<td>62.5</td>
<td>66.7</td>
</tr>
<tr>
<td>Volunteered</td>
<td>10</td>
<td>33.2 ± 4.7</td>
<td>40.0</td>
<td>60.0</td>
<td>10.0</td>
<td>20.0</td>
<td>70.0</td>
<td>70.0</td>
</tr>
</tbody>
</table>

Age: the age of the respondent; Birthplace: “Local” was a respondent who was inhabitant, or had spent the majority of his life, in villages in or in periphery of ABR, against “Outsider” who was coming from villages or towns ≥ 50 km of ABR borders; Education: respondent’s grade of education based on the Iranian education system, which was divided as “Primary” (low-illiterate or primary school), “Secondary” (lower, upper or post-secondary school), and “University” (High school diploma, pre-university or university degrees); Job: respondent’s employment status as either full-time contracts with permanent position (“Casual”) or part-time employed rangers (“Agency”); Experience: respondent’s years of working in ABR as a ranger; Patrol sections: number of patrol sections worked in ABR as a ranger out of the total eight sections. Guesstimates are combined median of rangers’ minimum and maximum perceived bear numbers for each patrol section among all the interviewed rangers in each group.

¹ “Total”: All the interviewed rangers; i.e., those rangers who agreed to participate in this survey (out of 26); “Volunteered”: interviewed rangers who volunteered to provide their perceptions of bear abundance in ABR for the entire study area (i.e. all the eight patrol sections).

² mean ± SD
Fig. 1. Map of the Arasbaran Biosphere Reserve (ABR), Iran, showing the spatial distribution of forest patches (gray polygons), human-dominated areas (darker gray polygons) along the Aras River, and locations of brown bear feces collected (crossed circles) within survey routes (solid black lines) across twenty-four 4.5 × 4.5-km cells (transparent gray). Upper inset map shows the location of the study area (black rectangle) in relation to the approximate geographical distribution of brown bears (dark gray) in southwestern Asia (redrawn from McLellan et al., 2017). Locations of the eight patrol sections and associated ranger stations (white flags) and core zones (darker grey polygons) that were used in the interview survey of local rangers are also shown in the lower inset map.
Fig. 2. Bayesian posterior density distribution for estimates of the density of brown bears in Arasbaran Biosphere Reserve (ABR), Iran, from Poisson-distributed spatial capture-recapture (SCR) model based on noninvasive genetic sampling of bear feces. Dotted line denotes the mode from the 150,000 total samples from the joint posterior distribution of density ($D$ in Table 1).
Fig. 3. Scatter plot of replicate versus actual discrepancy measures for the spatial capture-recapture (SCR) Poisson-distributed model used in this study. The Bayesian p-values are the proportion of points above the 1:1 equality line (black). $T_1$: Individual x trap frequencies, which summarizes the data by individual and detector specific Poisson counts aggregated over a single occasion. $T_2$: Individual encounter frequencies, which assess individual heterogeneity. $T_3$: Detector frequencies, which is based on aggregating over individuals and replicates to form detector-encounter frequencies.
Fig. 4. Rangers’ perceived abundance of brown bears in Arasbaran Biosphere Reserve (ABR), Iran, attributed to eight patrol sections (P1 to P8; see Fig. 1). The number of interviewed rangers who volunteered to provide bear guesstimates for each patrol section are shown in parentheses (range: 8-21 rangers). The edge line represents the median, the lower (dark gray) and upper (light gray) edges of the box are the first and third quartiles, and the whiskers the maximum and minimum points.
Supplementary Material

S1.1. DNA extraction, purification, and quality screening using mitochondrial analysis

All the procedures were done under a hood in a physically isolated room dedicated to low copy DNA samples. DNA extractions were performed using QIAamp DNA Stool Mini Kit (Qiagen Inc.) following the manufacture’s guideline with the following slight modifications: approximately 0.2 - 0.5 g of each fecal sample was soaked in ASL lysis buffer overnight (enough to cover the sample). Sample with buffer were then thoroughly mixed for 20 - 30 minutes and then put in a 70 °C-bath for 30 minutes. Fecal buffer were vortexed again for 5 - 10 minutes and 2 mL of this supernatant were used. All the stages were then the same as in the original instruction but we extended all the vortexing steps and digestion with Proteinase K following Skrbinšek et al. (2010). A negative control was included in each batch of 11 - 15 samples to control for contamination. Once not in use, DNA samples were stored at -20 °C.

To ensure that each sample contained sufficient amount of DNA, we performed a mitochondrial DNA analysis (Kohn et al., 1999). All fecal samples were initially screened using a carnivore-specific primer to amplify a 189-bp fragment of cytochrome b. This step helped to discard samples of very poor quality before DNA genotyping with microsatellites, so as to reduce costs and lab work. This step is described in details in Moqanaki et al. (2013).

We initially found that the extracted DNA in a portion of the samples with no success in the amplification attempts were not colorless, suggesting contamination with plant or diet materials in feces. To evaluate this hypothesis, we performed an inhibitor test. Accordingly, 0.1-10 µL volumes of eight randomly-selected colored DNA samples were separately added to a reaction volume containing one positive fecal-DNA to test if the suspicious extract blocks the reactions. As this test showed that PCR inhibitors were present, the problematic samples were purified with a Concentrated Chelex Treatment method described in Hebert et al. (2011). In brief, ca. 10 µL of 20% Concentrated Chelex was added to 20 µL of DNA sample and mixed briefly, and then the mixture
was boiled for 15 minutes. This mixture was then centrifuged at full speed for 5 minutes and the supernatant was extracted leaving the chelex in tube which then was discarded. Since this method appeared promising in recovering a portion of previously failed DNA samples, all the fecal DNA samples were purified and previously negative samples were re-amplified. DNA samples that successfully amplified were subjected to microsatellite analysis.

S1.2. Microsatellite genotyping, sex identification, individual identification

DNA genotyping for identifying individual bears was performed in two multiplex PCRs using eight previously published dinucleotide microsatellite loci and one sex determination locus (Table S1). Almost all these loci have recently been tested on another Caucasian bear population in Georgia and proved to be very informative (Murtskhvaladze et al., 2010). PCR conditions were from De Barba et al. (2010) and Skrbinšek et al. (2010) with minor changes. Final reaction volume of 7 µL consisted of 3.5 µL Qiagen multiplex mastermix, 0.7 µL Q-solution, 2 µL template DNA, 1 µL BSA, and ddH2O and forward (labeled) and reverse (unlabeled) primers to reach the appropriate concentrations in Table S1. PCR profile was the same for both multiplexes: an initial denaturation step of 95 °C/15', followed by a touchdown of 12 cycles at 94 °C/30", 57.3 °C/90" with a 0.4 °C-decrease in each cycle, 72 °C/60" followed by 27 cycles at 94 °C/30", 52.5 °C/90" and 72 °C/60", completed by a 30-min elongation step at 60 °C. In each reaction at least one negative and one reference bear DNA was included to monitor contamination and PCR efficiency, respectively. PCR products of each reaction (only one multiplex) were separated on a polyacrylamide gel and only products with clear DNA bands at ≥ 2 loci were considered for fragment analysis. Accordingly, 2 µL of the positive PCR products were diluted 10 times with ddH2O to balance signal intensity and 2 µL of this mixture were sent to Uppsala Genome Centre for sizing. In this step, each product was mixed with HiDi formamide and appropriate size standard and loaded on an ABI 3730XL DNA Analyzer (Applied Biosystems). We then analyzed and scored outputs using Geneious R6 software (ver. 6.1.6; Biomatters Ltd.).
**Table S1.** Primers for amplification of microsatellite loci and for sex determination of brown bear fecal samples used in this study in Arasbaran Biosphere Reserve (ABR), Iran (July 3 to September 17, 2012).

<table>
<thead>
<tr>
<th>Locus</th>
<th>5' primer</th>
<th>3' primer</th>
<th>C (µM)(^a)</th>
<th>Dye</th>
<th>Multiplex</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>G10B</td>
<td>GCCTTTTAATGTTCGTTGGAATTTG</td>
<td>GACAAATCACAGAAACCTCCATCC</td>
<td>0.08</td>
<td>HEX</td>
<td>1</td>
<td>PS</td>
</tr>
<tr>
<td>G10C</td>
<td>AAAGCAGAAGGCCTTGATTTTCTG</td>
<td>GTGGACATAAACACCGAGACAGC</td>
<td>0.1</td>
<td>HEX</td>
<td>1</td>
<td>P, J</td>
</tr>
<tr>
<td>G10P</td>
<td>ATCATAGTTTTACATAGGAGGAAAGAAA</td>
<td>TCGATGGGGAATACCTCTGAA</td>
<td>0.15</td>
<td>6FAM</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Mu11</td>
<td>AAGTAATTGGTGAATGACAGG</td>
<td>GAACCTCCACGGAAAATC</td>
<td>0.08</td>
<td>6FAM</td>
<td>1</td>
<td>T</td>
</tr>
<tr>
<td>Mu23(^b)</td>
<td>TAGACCAACAGGGCATCAG</td>
<td>TTGCTTGCTTAGACCCACC</td>
<td>0.08</td>
<td>HEX</td>
<td>2</td>
<td>BT</td>
</tr>
<tr>
<td>Mu59</td>
<td>GCTCCTTGGGACATTGTAA</td>
<td>TGACTGTCACCCAGGAG</td>
<td>0.15</td>
<td>HEX</td>
<td>2</td>
<td>BT</td>
</tr>
<tr>
<td>G10L</td>
<td>ACTGATTTTATTCACATTCCC</td>
<td>GATACAGAAACCTACCCCATGC</td>
<td>0.1</td>
<td>6FAM</td>
<td>2</td>
<td>BT</td>
</tr>
<tr>
<td>G10J</td>
<td>GATCAGATTTTTCAGCTTT</td>
<td>AACCCCTCACACTCCACCTTC</td>
<td>0.1</td>
<td>6FAM</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>SRY</td>
<td>GAACGCATTCTTGTGGTGTC</td>
<td>TGACTCGAGGTTTGACATTG</td>
<td>0.08</td>
<td>HEX</td>
<td>2</td>
<td>BT</td>
</tr>
</tbody>
</table>


\(^a\) Primer concentration for standard PCRs only. See the text for primer concentration for the two-step multiplex PCR.

\(^b\) Locus Mu23 showed an irregular repeat pattern and very weak readability, making reliable scoring of alleles not feasible. Therefore, we omitted this locus from the microsatellite genotyping, and used the remaining eight loci for subsequent analyses.
S1.3. Genotyping reliability and reducing genotyping errors

We carefully followed the recommendations by Bonin et al. (2004) to decrease errors during genotyping process. Microsatellite genotyping of noninvasive genetic samples are prone to certain errors, which can greatly influence estimation of population size (Creel et al., 2003; McKelvey and Schwartz, 2004). Errors are usually human-caused and may happen in every step, from sample collection to allele scoring (reviews in Pompanon et al., 2005; Beja-Pereira et al., 2009). Two main types of genotyping errors are allelic dropout (i.e., failure to amplify one allele in a heterozygous locus) and false alleles (i.e., misprinting of one allele) (McKelvey and Schwartz, 2004). As we expected relatively low amplification success and high genotyping error rates due to our sampling design, the criteria for dropping a poor quality sample was relaxed to not lose too many potentially informative, albeit work-demanding, samples (Lampa et al., 2013). We calculated the Probability of Identity (i.e. the probability that two randomly-chosen individuals from a population would have identical genotypes; $P_{ID}$) and $P_{ID}$ for siblings ($P_{SIB}$), which is a more conservative upper bound (Taberlet et al., 1999; Waits et al., 2001). The minimum number of autosomal loci necessary to obtain a $P_{SIB}$ was considered at $\leq 0.001$, so to have a high discrimination power to determine if two matching genotypes have originated from the same individual or full-siblings (Waits et al., 2001).

Meanwhile, we considered several screening steps and tested a two-step multiplexing method (Piggot et al., 2004). Since the multiplex pre-amplification method increases the cost and lab work, it is therefore advisable to test this method in a pilot study before applying it on large datasets (De Barba and Waits, 2010). We tested efficacy of the pre-amplification approach on our dataset by performing three independent amplifications on the low-quality extracts or genotypes with relatively high or high errors resulted from our initial conventional PCRs. If any improvement was observed, we performed two more amplifications until a sample could be either typed reliability or dropped from further analysis.

We typed each DNA sample at least three times and independent amplifications were performed up to 12 times to observe a heterozygous locus three times and one homozygous locus.
for 4 - 5 times. For sexing, we needed to observe the Y-related locus for at least two times to confirm a male, and no allele must be observed in at least three independent amplifications to confirm a female. We used RELIOTYPE (Miller et al., 2002) to assess reliability of our scores after typing each sample for at least three times, and conservatively accepted only genotypes with ≥ 95% reliability. The threshold was set at ≥ 99% for alleles that were observed in only one sample. We used GIMLET (version 1.3.3; Valière, 2002) to identify DNA samples with three or less allele mismatches. These samples were re-amplified up to three more times to evaluate their reliability.

S1.4. Two-step multiplex PCR amplification

Piggot et al. (2004) developed a method for amplification of low quality and quantity DNA samples. In this technique, samples are initially amplified in large volumes with all the primers in low concentration (“pre-amplification”). Then PCR product of this step is used as DNA for the second or “re-amplification” stage, usually with nested primers. On one hand, earlier studies have suggested that this technique substantially increases PCR success in noninvasive genetic samples (e.g., Bellemain and Taberlet, 2004; Hedmark and Ellegren, 2006; Lampa et al., 2008), yet more recent studies have questioned its significance (De Barba and Waits, 2010; Skrbinšek et al., 2010). On the other hand, Arandjelovic et al., (2009) have shown that although this method is very efficient in recovery of very low-quantity DNA samples (<25 pg), the use of nested primers does not significantly increase PCR success or decrease allelic dropout. Since the multiplex pre-amplification method increases the cost and lab work, it is therefore advisable to test this method in a pilot study before applying it on large datasets (De Barba and Waits, 2010).

We tested efficacy of the pre-amplification approach on our dataset by performing three independent amplifications on the low-quality extracts or genotypes with relatively high or high errors resulted from our initial conventional PCRs. If any improvement was observed, we performed two more amplifications until a sample could be either typed reliability or dropped from further analysis. PCR conditions and cycling regimes for the pre-amplification method were derived from
Skrbinšek et al. (2010) with slight modifications to adopt it to our multiplex combinations. We also did not use nested primers in the re-amplification step, as developed and suggested by Bellemain and Taberlet (2004), and the same primers in both stages were used. Pre-amplification was conducted in a final reaction volume of 20 µL: 10 µL of Qiagen Multiplex Mastermix, 2 µL of Q-solution, 5 µL of template DNA, 1 µL BSA, and 0.01 µM-concentration of each primer. All primers of each multiplex were typed together in this stage. PCR profile was: an initial denaturation at 95 °C/15’, followed by eight touchdown cycles of denaturation at 94 °C/30”, annealing at 62.4 °C/180” with a decreasing temperature of 0.3 °C in each cycle, and elongation at 72 °C/60”. The following regular PCR was 21 cycles at 94 °C/30”, annealing at 60 °C/180”, 72 °C/60”, and final elongation at 60 °C/30’.

The second-stage amplification (re-amplification) was performed in two multiplex PCRs using G10B/G10C and G10P/Mu11 for multiplex 1 and G10L/G10J and Mu23/Mu59/SRY for multiplex 2. The 7-µL reaction was consisted of 3.5 µL Qiagen Multiplex Mastermix, 0.7 µL Q-solution, 1.1 µL of PCR product from the pre-amplification stage, and 1.3 µL of water and primers to obtain 0.5 µM primer concentrations. PCR profile was the same for all multiplexes: initial denaturation at 95 °C/15’, followed by 12 touchdown cycles of denaturation at 94 °C/30”, annealing at 62.2 °C/90” with a decreasing temperature of 0.2 °C in each cycle, and elongation at 72 °C/60”. This was followed by 27 regular PCR cycles at 94 °C/30”, annealing at 60 °C/90”, followed by elongation at 72 °C/60”, and final elongation of 30 min at 60 °C. PCR products of each multiplex were then mixed in equal ratios, and 2 µl of this solution was diluted 20 times and 2 µL of this solution was used in allele sizing as described above.

To assess relative performance of the multiplex pre-amplification protocol on poor quality samples, amplification success and error rates of this method were compared to those of identical samples typed using the conventional PCR. The PCR amplification success was defined as number of successful PCRs out of the initial 3 attempts for each sample. Genotyping errors (i.e., allelic dropout and false alleles) were calculated in GIMLET (Valière, 2002) following the method proposed by

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Broquet and Petit (2004). GIMLET was used to identify independent DNA samples with matching autosomal genotypes (Valière, 2002).

References


Hebert, L., Darden, S.K., Pedersen, B.V., Dabelsteen, T., 2011. Increased DNA amplification success of non-invasive genetic samples by successful removal of inhibitors from faecal samples collected in the field. Conservation Genetics Resources 3(1), 41-43.


Table S2. Published mean estimates of home range sizes (km$^2$) of adult brown bears (M: male, F: female), including dispersing individuals, from neighboring populations to the Iranian Caucasus, with either 95% or 100% Minimum Convex Polygon (MCP) home range estimating methods.

<table>
<thead>
<tr>
<th>Population</th>
<th>Country</th>
<th>N</th>
<th>Sex</th>
<th>Age</th>
<th>Duration (days)</th>
<th>Home range size (km$^2$)</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>Turkey</td>
<td>2</td>
<td>F</td>
<td>Adult</td>
<td>$\approx$36-608*</td>
<td>14.07</td>
<td>95% MCP</td>
<td>AB</td>
</tr>
<tr>
<td>Caucasian</td>
<td>Turkey</td>
<td>5</td>
<td>M</td>
<td>Adult + Sub-adult</td>
<td>$\approx$36-608*</td>
<td>83.25</td>
<td>95% MCP</td>
<td>AB</td>
</tr>
<tr>
<td>Caucasian</td>
<td>Georgia</td>
<td>1</td>
<td>F</td>
<td>Adult*</td>
<td>*</td>
<td>25</td>
<td>100% MCP</td>
<td>L</td>
</tr>
<tr>
<td>East Balkan</td>
<td>Bulgaria</td>
<td>1</td>
<td>F</td>
<td>Adult</td>
<td>310</td>
<td>65.5</td>
<td>100% MCP</td>
<td>G</td>
</tr>
<tr>
<td>Dinaric-Pindus</td>
<td>Greece</td>
<td>1</td>
<td>F</td>
<td>Adult</td>
<td>330</td>
<td>57.6</td>
<td>100% MCP</td>
<td>M</td>
</tr>
<tr>
<td>Dinaric-Pindus</td>
<td>Greece</td>
<td>1</td>
<td>F</td>
<td>Adult + cub</td>
<td>157</td>
<td>255.08</td>
<td>100% MCP</td>
<td>M</td>
</tr>
<tr>
<td>Dinaric-Pindus</td>
<td>Croatia</td>
<td>5</td>
<td>F</td>
<td>Adult</td>
<td>561-914</td>
<td>58</td>
<td>100% MCP</td>
<td>HR</td>
</tr>
<tr>
<td>Dinaric-Pindus</td>
<td>Croatia</td>
<td>4</td>
<td>M</td>
<td>Adult</td>
<td>326-1330</td>
<td>128</td>
<td>100% MCP</td>
<td>HR</td>
</tr>
<tr>
<td>Alps</td>
<td>Slovenia-Italy</td>
<td>4</td>
<td>M</td>
<td>Adult</td>
<td>$\approx$153-335</td>
<td>358</td>
<td>100% MCP</td>
<td>K</td>
</tr>
<tr>
<td>Alps</td>
<td>Slovenia-Italy</td>
<td>4</td>
<td>M-peripheral</td>
<td>Adult</td>
<td>$\approx$214-396</td>
<td>1126</td>
<td>100% MCP</td>
<td>K</td>
</tr>
</tbody>
</table>

* Detailed information was not available.

References


Table S3. Variability of microsatellite markers used for individual multilocus genotyping of brown bear fecal samples collected in Arasbaran Biosphere Reserve (ABR), Iran.

<table>
<thead>
<tr>
<th>Locus</th>
<th>N</th>
<th>Size</th>
<th>$H_o$</th>
<th>$H_e$</th>
<th>$P_{ID}$</th>
<th>$P_{SIB}$</th>
<th>$P_{HWE}$</th>
<th>PCR</th>
<th>ADO</th>
<th>FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>G10B</td>
<td>8</td>
<td>139 - 163</td>
<td>0.61</td>
<td>0.68</td>
<td>0.09</td>
<td>0.40</td>
<td>0.4947</td>
<td>0.83</td>
<td>0.135</td>
<td>0.164</td>
</tr>
<tr>
<td>G10C</td>
<td>9</td>
<td>96 – 115</td>
<td>0.95</td>
<td>0.82</td>
<td>0.05</td>
<td>0.35</td>
<td>0.9410</td>
<td>0.88</td>
<td>0.033</td>
<td>0.259</td>
</tr>
<tr>
<td>G10P</td>
<td>8</td>
<td>168 - 182</td>
<td>0.91</td>
<td>0.79</td>
<td>0.07</td>
<td>0.37</td>
<td>0.9974</td>
<td>0.71</td>
<td>0.118</td>
<td>0.115</td>
</tr>
<tr>
<td>Mu11</td>
<td>8</td>
<td>78 – 93</td>
<td>0.84</td>
<td>0.78</td>
<td>0.08</td>
<td>0.38</td>
<td>0.9102</td>
<td>0.85</td>
<td>0.116</td>
<td>0.090</td>
</tr>
<tr>
<td>G10J</td>
<td>7</td>
<td>86 – 99</td>
<td>0.77</td>
<td>0.81</td>
<td>0.06</td>
<td>0.35</td>
<td>0.2761</td>
<td>0.56</td>
<td>0.133</td>
<td>0.018</td>
</tr>
<tr>
<td>Mu59</td>
<td>12</td>
<td>98 – 127</td>
<td>0.95</td>
<td>0.85</td>
<td>0.04</td>
<td>0.33</td>
<td>0.2489</td>
<td>0.55</td>
<td>0.084</td>
<td>0.012</td>
</tr>
<tr>
<td>G10L</td>
<td>9</td>
<td>136 - 162</td>
<td>0.89</td>
<td>0.85</td>
<td>0.04</td>
<td>0.33</td>
<td>0.6821</td>
<td>0.57</td>
<td>0.086</td>
<td>0.016</td>
</tr>
<tr>
<td>Mean</td>
<td>8.6</td>
<td>0.85</td>
<td>0.80</td>
<td></td>
<td>1.981e-09</td>
<td>0.0008</td>
<td>0.9284</td>
<td>0.71</td>
<td>0.101</td>
<td>0.087</td>
</tr>
</tbody>
</table>

N: the number of observed alleles; Size: allele size range (bp); $H_o$: observed heterozygosity; $H_e$: expected heterozygosity; $P_{ID}$: the unbiased probability of identity; $P_{SIB}$: the unbiased probability of identity among siblings; $P_{HWE}$: the probability of the data under the assumption of the null hypothesis of Hardy–Weinberg equilibrium; PCR: mean value of PCR success rate; ADO: the rate of allelic dropout; FA: the rate of false alleles.

References for microsatellite markers are in Table S1, Supplementary Material.
Fig S1. Effect of the length of survey (m) on brown bear basal probability of detection, based on genotyped bear feces collected in Arasbaran Biosphere Reserve (ABR), Iran. The solid black line shows the posterior mean, and the grey lines show the relationships based on a random posterior sample of size of 200 to visualize estimation uncertainty.