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Wolf survival and population trend using non-invasive capture–recapture techniques in the Western Alps

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Summary

1. Reliable estimates of population parameters are often necessary for conservation management but these are hard to obtain for elusive, rare and wide-ranging species such as wolves *Canis lupus*. This species has naturally recolonized parts of its former habitat in Western Europe; however, an accurate and cost-effective method to assess population trend and survival has not been implemented yet.

2. We used open-model capture–recapture (CR) sampling with non-invasive individual identifications derived from faecal genotyping to estimate survival and trend in abundance for wolves in the Western Alps between 1999 and 2006. Our sampling strategy reduced individual heterogeneity in recaptures, thus minimizing bias and increasing the precision of the estimates.

3. Young wolves had lower apparent annual survival rates (0·24 ± 0·06) than adult wolves (0·82 ± 0·04); survival rates were lower in the summer than in the winter for both young and adults. The wolf population in the study area increased from 21 ± 9·6 wolves in 1999 to 47 ± 11·2 wolves in late winter 2005; the population growth rate (λ = 1·04 ± 0·27) was lower than that recorded for other recolonizing wolf populations.

4. We found a positive trend in wolf abundance, regardless of the method used. However, the abundance estimate based on snow-tracking was on average 36·2% (SD = 13·6%) lower than that from CR modelling, because young dispersing wolves are likely to have lower sign detection rates in snow-track surveys, a problem adequately addressed by CR sampling.

5. *Synthesis and applications*. We successfully implemented a new method to assess large carnivore population trend and survival at large spatial scales. These are the first such estimates for wolves in Italy and in the Alps and have important management implications. Our approach can be widely applied to broader spatial and temporal scales for other elusive and wide-ranging species in Europe and elsewhere.

Key-words: Alps, *Canis lupus,* capture–recapture, genetic, monitoring, population size, survival, wolves

Introduction

Population abundance and related parameters need to be assessed to implement effective wildlife management. These essential parameters are often very hard to obtain for rare, wide-ranging and elusive species, particularly those listed as endangered or threatened (IUCN 2001). In Italy, wolves *Canis lupus* Linnaeus 1758, now a fully protected species in Western Europe, recently recolonized the Western Alps after 70 years through dispersal from the Apennines (Fabbri *et al*. 2007). This wolf population is expanding (Boitani 2003), although accurate estimates of trends of population size and survival are lacking.

Large carnivores like wolves are difficult to study because they are often wide-ranging and at low densities (Boitani 2003). Fuller, Mech & Cochrane (2003) identified knowledge gaps for future wolf research efforts, including the development of accurate and cost-effective methods for assessing wolf

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abundance to monitor wolf recovery and control actions. In the past, wolf monitoring programmes have relied on physical captures, collaring and radiotelemetry, a method that is logistically difficult and expensive to implement (Hayes & Harestad 2000; Fuller *et al.* 2003). Moreover, these and other techniques, such as field observations, trapper questionnaires, extrapolations of habitat suitability to estimate abundance, ground-based telemetry studies or howling surveys can only approximate or index the regional population size but lack the rigour of formal estimates of population size that address the problem of imperfect detections during surveys (Fuller *et al.*1992; Boitani 2003; Williams, Nichols, & Conroy 2002) and are poorly suited to assess population trends (Garshelis & Hristienko 2006).

Non-invasive survey methods to assess population parameters in elusive, wide-ranging carnivores are increasingly used under a capture–recapture (CR) modelling framework involving 'captures' of individual carnivores from camera trap photos (Karanth *et al.* 2006) or based on DNA from field-collected scats (Creel *et al.* 2003; Prugh *et al.* 2005; Meijer *et al.* 2008). Genetic markers from scats can then be used to track the fate of individuals over long periods (Creel *et al.* 2003; Lukacs & Burnham 2005). CR survey methods can then be employed to accurately estimate capture probabilities, population size and trend, and survival rate (Nichols 1992).

We used a genetic CR approach to monitor wolf population size over 7 years (1999–2006) and estimate survival rates in the Western Alps. We developed a sampling design based on large-scale monitoring of snow-tracks to derive minimum counts of wolves as well as to collect scats in a manner that accounted for the effects of differential marking behaviour of wolves to minimize individual heterogeneity in recaptures. Additionally, we investigated factors that could increase the probability of creating false individual genetic identities through genotyping errors. Finally, we applied open population CR models to long-term faecal genotyping data to estimate wolf survival rates and population trends in the Alps.

Materials and methods

STUDY AREA

The study area encompasses *c.* 4000 km^2 of the Ligurian, Maritime, and Cozie ranges of the Western Alps in Italy and France (Fig. S1). The core area is characterized by long narrow valley bottoms surrounded by rugged mountains, with elevations ranging from 700 to 3842 m. Dense coniferous and broadleaf forests cover about 50% of the study area; the remainder consists of alpine meadows and shrubland/rock areas. The snow-season generally lasts from October–November to April–May.

SAMPLING DESIGN FOR SCAT COLLECTION

Scat collection and sampling sessions

A few days after a snowfall, we conducted ski- or snowshoe-based surveys with teams of 3–20 people searching for wolf tracks along transects that systematically monitored the entire study area. When we found wolf tracks, we followed them as long as possible and collected every scat along the track. We covered all transects one to seven times during each of two winter sessions: early winter (EW) from October to January and late winter (LW) from February to May. We also opportunistically collected fresh scats when encountered during other field activities. We stored scats at −30 °C until each was transferred to a test tube containing either 95% ethanol or silica gel. We non-randomly selected a subsample of scats for the genetic analysis due to funding limitations. We prioritized fresh scats to optimize laboratory success (Lucchini *et al.* 2002), groups of scats found along the same wolf track to increase the probability of characterizing each individual in the pack, and single scats found where the presence of a wolf pack was not documented.

We assessed effort in two ways:

1. 'Field effort', or the sampling effort per area (Fig. S1), was expressed as: Average number of surveys per session in each area × Average number of people per survey in each area. A low effort category was defined when this value was ≤60, and high effort was defined when this value was > 60.
2. 'Laboratory effort' was expressed as: Analysis rate (i.e. the percentage of the collected samples that were analysed in the laboratory) × success rate (i.e. the percentage of the samples analysed in the laboratory that gave positive multilocus genotypes) for each session within each year. These values were standardized by subtracting the mean and dividing by the standard deviation.

Analysis of marking behaviour

We documented the marking behaviour of wolves related to each collected scat following Vila *et al.* (1994). We reported the location of each scat (i.e. on trail/road, off trail), the position (e.g. on bush, on rock, on the ground, etc.), and the distance to junctions, passes, ridges and ecotones. Scats located on trails and roads, on a higher substratum, or near (< 40 m) a junction, pass, ridge or ecotone, represent strong marking behaviour (Vila *et al.* 1994). We assigned a unique marking behaviour value (UMB) to each scat. Scats received one point for each documented strong marking behaviour. Points were summed to give the UMB value to each scat. Higher UMB values correspond to a more intense marking behaviour, and therefore scats more likely to be detected (e.g. scats on trail/road vs. scats off trail). We calculated the percentage of non-marking scats (i.e. UMB value of zero) and in particular of 'off trail scats', to check the extent to which we could collect these harder to detect scats using our sampling technique. We evaluated if the age (adult or young, see below) of the wolf which produced the scat was independent from the UMB value of the scat, or in particular from the location of the scat (i.e. on trail/road, off trail), using χ^2 tests of independence.

GENETIC LABORATORY ANALYSIS

DNA analysis and error checking protocol to reduce genotyping errors

DNA extractions, microsatellite and mtDNA analysis, sex determination, and probability of identity analyses are described in Appendix S1 in Supporting Information. We minimized the probability of creating a new individual through genotyping errors using a combined approach based on protocols by Taberlet *et al.* (1996), Paetkau (2003), McKelvey & Schwartz (2004, 2005) and Kalinowski *et al.* (2006), and on the use of independent field information. To detect

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errors, each scat was amplified three times at each locus using a multi-tube polymerase chain reaction (PCR) approach (Taberlet *et al.* 1996) and scored by two independent observers. Samples that did not amplify, or were inconsistent between the three amplifications, were re-extracted and re-amplified. Secondly, we grouped identical and near identical genotypes [i.e. samples that differed only by one or two mismatches (MM) – Paetkau 2003] using the program GIMLET (Valiere 2002); these were the most likely to contain errors (Prugh *et al.* 2005). Every such sample was systematically reanalysed. Thirdly, every confirmed genetic result was compared to data from the track followed, direct observation data, dead recovery data, or radiotelemetry data for independent field confirmation. We conducted additional extractions and amplifications for samples which produced data incongruent with field information. Fourthly, we conducted the EB test and the DCH test (McKelvey & Schwartz 2004) using program DROPOUT (McKelvey & Schwartz 2005). The process of iteratively re-running samples, evaluating the results with independent field data, and executing the EB and DCH test was continued until no errors were detected. We defined the type of error detected per locus (scoring errors, false alleles, dropouts, partial null alleles) and counted the number of individual wolves removed. After error removal, we used ML-RELATE (Kalinowski *et al.* 2006) to test for the presence of null alleles at each locus as indicated by deficiency of heterozygotes relative to Hardy–Weinberg expectations.

Parental relationships and age determination

We used field observations, such as information from radiocollared animals, pack membership derived from snow-tracking information, the recoveries of dead individuals of which it was possible to determine reproductive status and age, to derive and test specific relationship hypotheses among individuals using the software ML-RELATE (Kalinowski *et al.* 2006). Within each pack and each year, we evaluated the range of relationships possible for the genotypes detected – unrelated, half-sibling, full-sibling, and parent-offspring – and identified the relationship with the highest likelihood (Wagner, Creel, & Kalinowski 2006). We determined for each pack-year if the wolf detected was a parent, an offspring, or an immigrant with no relationships with other members of the pack. If it was not possible to classify the wolf into one of these categories, the wolf status was considered unknown. We then defined three age classes: adults (wolves > 1 year old, which included parents that year and all the individuals present in the study area the year before); young (wolves < 1 year, which were the documented offspring of the pack that year); or unknown (wolves for which we did not know the starting age).

CR ESTIMATION OF POPULATION PARAMETERS

Each wolf scat successfully analysed was considered a 'capture or recapture.' We constructed a capture history for each wolf by recording whether it was sampled in each sampling session. These capture histories were imported into program MSURGE 1·8 for analysis (Choquet *et al.* 2004). We used the open population, Cormack–Jolly–Seber (CJS) model, which estimated apparent survival (Φ) and recapture rates (p) of wolves. Following recommendations by Lebreton *et al.* (1992), we checked the goodness-of-fit of a fully parameterized model using program UCARE 2·2 (Choquet *et al.* 2005). The overall test is composed of tests for transience and trap-dependence (trap-happiness or trap shyness), which are particularly sensitive to heterogeneity in recaptures and independence of individuals (Pradel *et al.* 1997). We defined a set of 20 biologically plausible models, where survival probabilities were modelled to examine variation over the year (summer and winter), age (young, adults, or unknown) and sex; and recapture probabilities were modelled as a function of years or winter seasons (EW and LW), to examine the importance of years/seasons, age, sex and effort (laboratory and field). Laboratory effort was used as a time-dependent external covariate in a logit-linear relationship to recapture probability. Models were ranked and weighted according to the Akaike information criterion (AIC_c, Burnham & Anderson 1998). We model averaged the best models with $\Delta AIC_c < 2$ to obtain parameter estimates and standard errors (Burnham & Anderson 1998). We estimated standard errors of the annual survival rates using the Delta Method (Seber 1982). We evaluated the importance of each covariate in explaining the parameter of interest and we measured this importance by the sum of Akaike weights over the subset of models that included that variable (Burnham & Anderson 1998).

We used the estimates of recapture rates from the best CJS model to estimate population size [using a Horvitz-Thompson-type estimator $[\hat{N}_t]$ (e.g. McDonald & Amstrup 2001)] and variance. This estimator corresponds to the canonical estimation approach which is a count statistic divided by the corresponding estimate of detection probability (Williams *et al.* 2002). We also estimated population size and empirical confidence intervals using a bootstrap approach with 500 replicates and program MSURGE made available by R. Choquet (unpublished data).

We compared the snow-tracking minimum estimates of population size, the counts of simultaneous genotypes, the explicit CR estimates of population size and the bootstrap CR estimates for each season. We estimated the averaged finite rate of increase (λ) from late winter to the following late winter as the ratio of the population sizes during successive winters.

Results

SCAT SAMPLING

Transects were searched for wolf tracks; once found, we followed a total of 3366 km of wolf tracks and collected 3382 wolf scats over the 14 sampling sessions (seven early and seven late winter sessions). We genotyped 1399 scats (a 41·4% analysis rate) and identified 87 unique individual genotypes from the 739 scats that provided successful results (a 52·8% laboratory success rate). Analysis rates per sampling session ranged from 12·9% to 79·4%; laboratory success rates ranged from 15·4% to 77·8%. The measure of 'laboratory effort' varied between sampling sessions from −1·21 to 2·23. The number of captures (i.e. scats) per individual ranged from 1 to 56; 31·0% of the individuals were captured only once. We pooled the captures in each session, and considering the sampling sessions, 43·7% of the individuals were captured only once.

Analysis of marking behaviour

We documented the marking behaviour for 489 successfully genotyped scats. Of these, 35·2% were collected off-trail and 16·0% had a UMB of 0. These two percentages represent the non-marking scats, harder to detect and collect. Of the successfully genotyped scats, 26·6% were from young wolves. However, young wolves comprised 54·0% of the individuals

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sampled, which indicates that young wolves were re-sampled fewer times than adults. Scat from young wolves were more often found off-trail (54·6% vs. 28·1% for adults; $\chi^2 = 29{\cdot}4$, d.f. = 1, $P << 0{\cdot}001$) and more likely had a UMB of 0 (24·6% vs. 12·8% for adults; $\chi^2 = 20{\cdot}3$, d.f. = 5, $P = 0{\cdot}001$). If we had not sampled off-trail, we would have missed 14·9% of the individuals, all in the young category, which corresponded to 27·7 % of the total number of young wolves.

GENETIC ANALYSIS

Genotyping error rates and probability of identity

The average expected heterozygosity of the 10 loci was 0·62 (range = 0·50–0·76; SD = 0·10); the average number of alleles per locus was 3·6 (range = 3–5; SD = 0·70). The observed P_{ID} was 2·10e-05, computed using up to five loci. The theoretical $P_{ID\text{-}sib}$ was 1·50e-02 with six loci. After conducting multiple error-checking approaches, we eliminated 46·3% of the initially estimated number of individuals obtained after the multitube approach. We ultimately obtained 87 high quality genotypes. The number of replicates conducted per sample per locus ranged from 3 to 19. Of the errors detected, 55·0% were due to allelic dropouts, 17·1% were due to false alleles, 25·7% were due to partial null alleles and 2·2% were due to scoring or typing errors. The majority of errors were produced by genotypes that were sampled only once (85·3%) and differed only by one allele in one locus (71·6%) from the real genotype; these were easily identified by inconsistency with field information. We also detected samples that produced multiple errors (18 samples produced 2-MM pairs, 6 produced 3-MM pairs and 1 produced 4-MM pairs), that were detected because they were only sampled once, and/or were inconsistent with field information. The most problematic error we detected was a systematic error that led to multiple recaptures of false individuals, probably due to the presence of a partial null allele in locus *cph2*. DCH tests confirmed the removal of errors. We found no heterozygosity deficiency after error removal (H–W tests: $0{\cdot}2 < P$-values $< 0{\cdot}9$ for the 10 loci), or null alleles in any locus ($P_n < 0{\cdot}0001$), except for locus fh2137 where P_n was 0·015, which can be considered negligible (Kalinowski *et al.* 2006).

Parental relationships, social status, and age determination

We identified 87 unique individuals: 39·1% were females and 51·7% were males (9·2% were of unknown sex). We defined pack membership for each individual and documented seven wolf packs, three solitary wolves with permanent territories, and 18 dispersers of which 6 dispersed outside the study area and 12 within the study area. We were able to reconstruct detailed relationships and identified the reproductive pair every year for five packs. Most (54·0% of the individuals) were young wolves when first sampled (they were young only for the first year of sample, then transitioned to adults), 19·6% were adults when first sampled, and the rest were of unknown age.

WOLF POPULATION TREND AND SURVIVAL

The overall goodness-of-fit test was not significant ($\chi^2 = 8{\cdot}911$, $P = 0{\cdot}998$) and showed no sign of transience ($z = 0{\cdot}595$, 2-sided test, $P = 0{\cdot}552$) nor of trap-dependence ($z = -0{\cdot}672$, 2-sided test, $P = 0{\cdot}501$), suggesting that the fully parameterized CJS model fit the data well. In particular, heterogeneity among individuals in detection probability was negligible. We then examined reduced-parameter models: the three best models with $\Delta AIC_c < 2$ included age and season as additive effects, affecting apparent survival (Φ) and the two measures of effort (field and laboratory) as main effects with an

Table 1. Top 15 Cormack–Jolly–Seber models run in MSURGE to calculate maximum likelihood estimates of recapture rates (*p*) and apparent survival rates (Φ) of wolves in the Western Alps, 1999–2006.

Model	AIC_c	ΔAIC_c	AIC_c weights	No. parameters
p (FE × LE) Φ (age + season)	463·27	0	0·36	7
p (FE + LE) Φ (age + season)	464·30	1·03	0·21	6
p (FE) Φ (age + season)	464·88	1·61	0·16	5
p (FE) Φ (age × season)	465·93	2·66	0·09	6
p (FE) Φ (3 age classes + season)	465·95	2·68	0·09	6
p (FE + t) Φ (age + season)	467·71	4·44	0·04	17
p (FE + age) Φ (age + season)	468·30	5·03	0·03	7
p (FE all areas) Φ (age + season)	469·59	6·32	0·02	11
p (FE) Φ (season effects three age classes)	474·42	11·15	0·00	7
p (FE) Φ (season effects young)	475·42	12·15	0·00	6
p (FE) Φ (age)	477·55	14·28	0·00	5
p (season) Φ (age + season)	479·77	16·5	0·00	5
p (LE) Φ (age + season)	480·15	16·88	0·00	5
p (age) Φ (age + season)	481·35	18·08	0·00	6
p (age + season) Φ (age + season)	483·57	20·3	0·00	7

Models are ranked according to their AIC_c values.
AIC, Akaike information criterion; FE, field effort; LE, laboratory effort.

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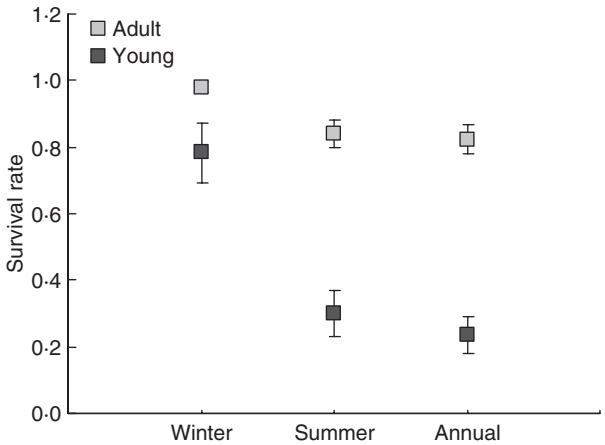

Fig. 1. Estimated apparent survival rates for young and adult wolves in the Western Alps, 1999–2006. Error bars represent 95% CI. These maximum likelihood estimates were obtained by averaging the three best Cormack–Jolly–Seber (CJS) models.

interaction affecting recapture rate (p) (Table 1). All the top models included age and season as covariates for apparent survival either as additive effects or in interaction with the other effects. In all models, age was best explained if young and unknown, two of the categories for age, were considered equal. The sum of the Akaike weights for age and season was 1·00, which indicates the high importance of these two variables in explaining probability of survival. Young wolves had a lower annual survival rate (0·24 ± 0·06) than did adults (0·82 ± 0·04) (Fig. 1). Survival rates were lower in the summer than in the winter for both young and adults (Fig. 1). Recapture rates were best explained by field effort (the sum of Akaike weights for the covariate field effort was 1·00, Table 1); the weight for laboratory effort was 0·57, indicating a lower importance (Table 1). Sex had a minimal effect and was, therefore, absent from the top 15 models (Table 1). Recapture rates varied from 0·28 ± 0·12 to 0·77 ± 0·07 depending on field and laboratory effort.

Population size derived from the Horvist-Thompson estimator increased from 1999 to 2005 and decreased during the last winter 2005–2006. Abundance was generally greater during early winter than late winter (Fig. 2). The log mean of the back-transformed estimates using bootstrapping followed the same trend (Fig. 2). The two types of estimates of log population size were, as expected, highly correlated (r = 0·9416, P < 0·001) (Figs 2 & 3). Log transformation of estimated population size was sufficient to provide a more stable estimator, and had the further advantage of stabilizing the variance as well (Seber 1982) (Fig. 3). Similar results were obtained by discarding all estimates that were higher than 500 from the calculation of the mean bootstrap estimate (i.e. constrained mean in Fig. 3). The bootstrap estimates had a distribution strongly skewed to high values, and the resulting confidence intervals were thus not symmetrical, while those derived from the maximum likelihood estimates and their associated standard errors estimates were by definition symmetrical. The bootstrap confidence intervals were thus expected to be more reliable (i.e. to encompass a coverage closer to the expected one, the unknown true value). We detected a positive trend in wolf abundance regardless of which method was used (Fig. 2), though snow-tracking data suggested fewer wolves than did CR modelling (Fig. 2). The number of packs detected varied less between EW and LW than population size (Fig. 2). The average LW to LW finite rate of increase (λ) of the population was 1·04 ± 0·28. The high variation is largely due to the last year estimate when the population declined in size.

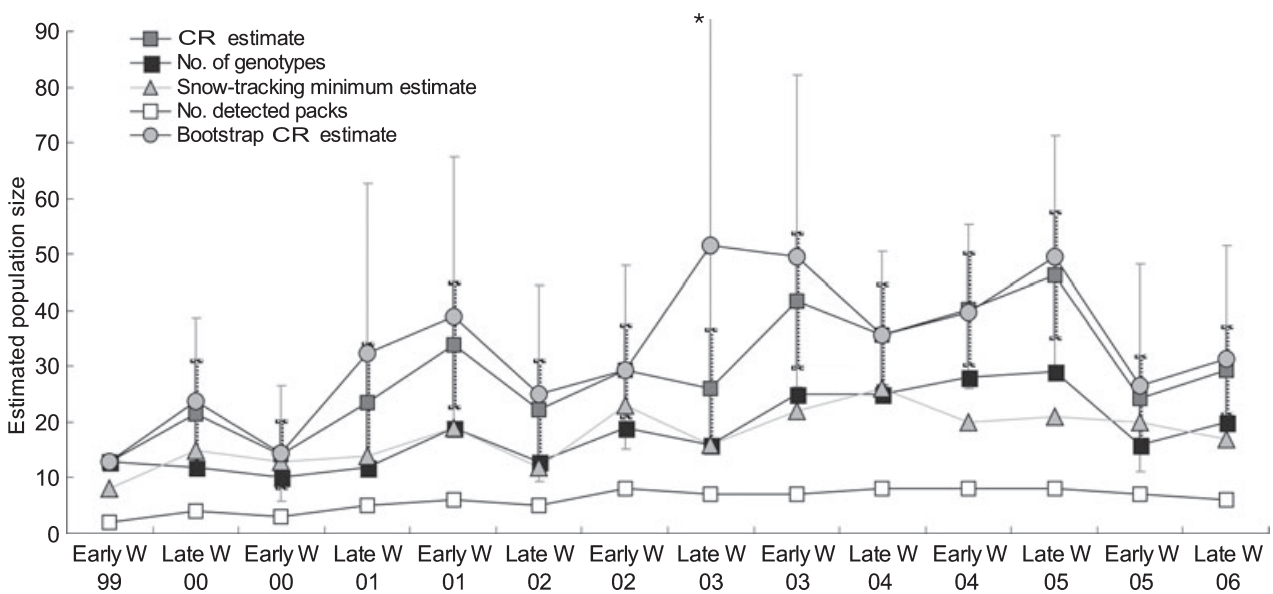

Fig. 2. Total estimated wolf population size in the Western Alps from the early winter 1999 (Early W 99) to the late winter 2006 (Late W 2006). Population size was estimated using CR (±SE), the bootstrap method (and 95% CI), the maximum number of genotypes detected, and the snow-tracking minimum estimate. *The top of the CI is 122.

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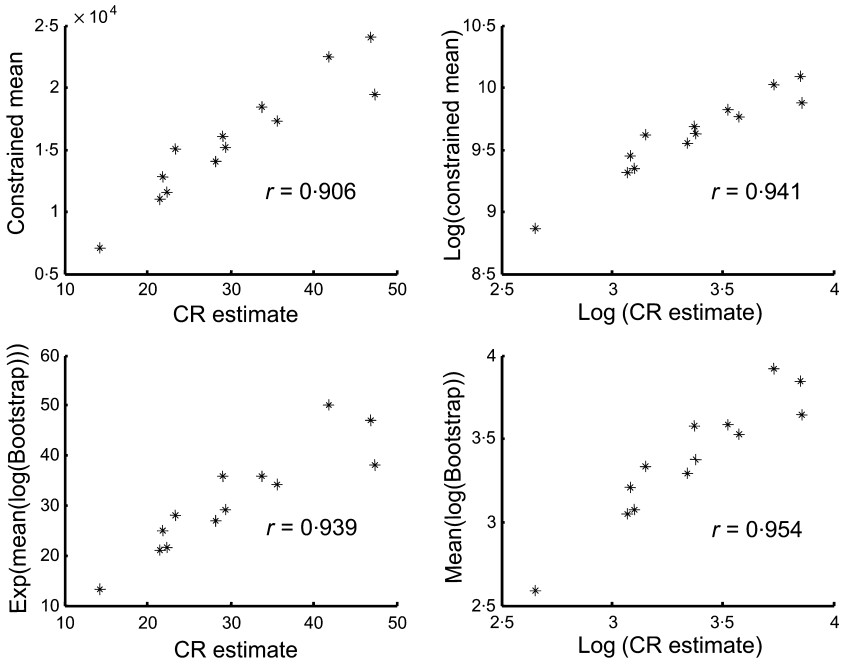

Fig. 3. Correlation between standard CR population size estimates and bootstrap mean estimates of wolf population abundance in the Western Alps (1999–2006). The constrained mean was obtained discarding (for each season) all estimates that were higher than 500 from the calculation of the mean bootstrap estimate.

Discussion

WOLF POPULATION TREND AND SURVIVAL IN THE WESTERN ALPS

We successfully implemented a new, non-invasive and large-scale, CR approach to produce the first wolf survival and precise population size estimates in the Alps of Western Europe. The wolf population increased over the 7 years of our study, as did the number of packs observed. All five methods we used supported this trend, though we estimated fewer wolves every season with snow-tracking data than we did by CR modelling. Packs are the primary social unit of a wolf population, but the population includes lone wolves – typically dispersers, too (Fuller *et al.* 2003). Genetic CR modelling is more likely to account for these individuals than is snow-tracking counts, because young dispersing wolves are likely to have lower sign detection rates in snow-track surveys, a problem adequately addressed by CR sampling. Genetic CR population size estimates should always be corroborated with estimates of the number of packs and with survival rates. These estimates are more robust to heterogeneity in recaptures (Lebreton *et al.* 1992), more likely to correctly interpret population trends, and more likely to identify solitary wolves. This is especially true in newly recolonized areas, such as the Alps. In fact, the majority of the population in our study is composed of young wolves, which is typical of wolf recolonization in areas with plentiful suitable habitat (Fuller *et al.* 2003). Lower abundance estimates occur during LW, as expected based on the life cycle of wolves; hence, the late winter estimate is probably more conservative. Similar results have been found in North American wolf populations (Pletscher *et al.* 1997; Fuller *et al.* 2003).

Survival rates of young in the Alps were lower than in similar recolonizing situations (e.g. Pletscher *et al.* 1997), and this probably explains the lower lambda we found in Western Europe compared to those found in other studies (e.g. Hayes & Harestad 2000). The wolf population suddenly decreased during winter 2005–2006, perhaps due to poaching (we documented illegal poisoning of two radio-collared wolves); this population is still very low and its continued existence is not assured. Apparent survival rates were best explained by age and season. Young wolves had much lower survival rates than adults, and summer survival was lower than winter survival. Winter apparent survival for young is virtually true survival, because dispersal is very unlikely during the first 10 months of life (Boyd & Pletscher 1999). It is impossible to distinguish between dispersal outside the study area and mortality during the summer. High turn-over of individuals within packs was documented for young, whereas the adults and the alpha pairs generally maintained their position for several years. Experience probably enabled them to survive in an ecosystem with high human presence.

RECOMMENDATIONS FOR NON-INVASIVE CR MONITORING STUDIES

Genetic CR techniques are a promising tool to estimate population parameters and monitor populations through

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time (Schwartz, Luikart & Waples 2007) for a variety of species and locations (e.g. Boulanger *et al.* 2004). However, limitations and specific sources of error are present using a non-invasive dataset in a CR framework (Creel *et al.* 2003; Lukacs & Burnham 2005). The presence of capture heterogeneity is one of the most difficult problems facing estimation of animal abundance using CR (Pledger & Efford 1998). Problems of capture heterogeneity with wolves can arise during scat sampling due to differing behaviour of individuals leading to disparate probabilities of finding their scats. For example, young wolves were more likely to be sampled with scats collected off or away from human trails than at marking sites. Young and solitary wolves usually are subordinates which do not intensively mark the territory (Vila *et al.* 1994). In fact, we would have missed 27·7 % of young wolves if we simply sampled on roads or human trails. Therefore, a sampling design based on collection of scats along wolf tracks increases the probability of characterizing each individual, especially young and dispersing wolves. With this design, we produced a dataset which met the assumptions of homogeneity in recapture rates which is fundamental for CR modelling. The use of scat-detection dogs (Smith *et al.* 2005) may aid in the detection of non-marking scats during the summer or in areas where snow is never present. The probability of detection (i.e. recapture rates) did not decrease with an increase in population size through time; in fact, time was not an important covariate in explaining recapture rates in the CJS models. Furthermore, the estimates of population size did not increase with increases in sample size. These two indices are good, indirect evidence of the absence of genotyping errors in the dataset. We suggest acknowledging that genotyping errors might not be completely eliminated from any single laboratory protocol, and then working with a multiple approach to obtain the most reliable results for subsequent CR analysis. We suggest that the best checks on laboratory errors is through the use of independent field data and the re-examination of any samples which produce results incongruous with field information. Open CR models such as CJS are best applied over multiple years to document population trends. These models are flexible because they do not assume a closed population or a strict sampling design, are well suited for non-invasive samples from elusive or rare species, and in some cases facilitate modelling of capture heterogeneity in a biologically relevant manner (Lebreton *et al.* 1992). The overall procedure (selection of a survival-recapture parameter structure for the marked individuals, then application of a Horvitz-Thompson estimator) amounts to use of the Jolly-Seber model, with properly constrained survival and recapture parameter structure. Our implementation has the advantage of easily providing bootstrapped confidence intervals, more reliable than the usual parametric confidence intervals for population size, because of the strong and well known asymmetrical distribution of population size estimates based on capture–recapture (Seber & Manly 1985).

RECOMMENDATIONS FOR MANAGEMENT

The wolf population in the Alps has been identified as a unique population segment (Linnell, Salvatori & Boitani 2007), connected demographically (Marucco *et al.* 2007) and genetically to the Italian wolf population in the Apennines (Fabbri *et al.* 2007), but different in ecological and socio-economic contexts (Linnell *et al.* 2007). Furthermore, it is a population that is currently recolonizing a considerable area in Italy, France and Switzerland. The population trend and survival rates we estimated are necessary to inform management decisions and to understand the dynamics of this new alpine wolf population. PVA models have provided important insights on how to manage the processes threatening species of conservation concern and should rely on precise life-history data from the population of interest; however, researchers are often forced to make assumptions when such data are missing (Ellner *et al.* 2002). Chapron *et al.* (2003) based a wolf population modelling analysis, developed to design wolf management strategies in the Alps, on survival estimates from North American studies. The apparent survival probabilities we estimated here could be used in this framework to improve those analyses. Wolf recovery is continuing throughout the Alps, therefore, the approach described here will be fundamental in monitoring the population in the future. The sampling design for this monitoring programme should consider a lower field effort than the present study by avoiding multiple encounters of an individual within a sampling session. Recaptures within sessions cannot be used and were lumped together into single detections. Our approach can also be widely applied to broad spatial and temporal scales for other elusive and wide-ranging species in Europe and elsewhere.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Genetic laboratory analysis

Fig. S1. The Western Alps study area.

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