SPATIAL POPULATION DYNAMICS OF RECOLONIZING WOLVES IN THE WESTERN ALPS

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The wolf recolonized part of its former habitat in the South-Western Alps through dispersal from the Apennines beginning in the late 1990s. Prioritization of conservation actions and effective management of this species relies on estimates of wolf population trend, survival rates, occupancy parameters, and on the development of a spatially explicit population model. These estimates were lacking in Italy and Western Europe, and accurate and cost effective methods to assess these parameters have not been implemented.

I used genetic capture-mark-recapture techniques with long-term fecal genotyping data and applied open-population models to estimate survival rates and assess trend in abundance of wolves in the Western Alps. The wolf population in the study area increased from 21 ± 9.6 wolves in 1999 to 47 ± 11.2 wolves in late winter 2005. Young wolves (< 1 year-old) had lower apparent annual survival rates (0.24 ± 0.06) than adult wolves (0.82 ± 0.04).

I applied an unconditional multi-season occupancy model to estimate wolf occupancy dynamics. Human disturbance ($\beta = -5.553$, SE = 2.186) and rock-area cover ($\beta = -4.129$, SE = 1.392) had negative effects on occupancy, while the presence of red deer ($\beta = 0.694$, SE = 0.306) and forested-area cover ($\beta = 0.596$, SE = 0.458) had positive effects. The wolf recolonization process was characterized by a Markovian change in occupancy and the sites were not in an equilibrium state. This is typical of an expanding population.

The habitat suitability map produced from the occupancy analyses was fundamental for the development of a spatially explicit, individual-based model which allowed a full analysis of this complex spatial and temporal wolf recolonization of the Italian Alps. I predicted wolf pack numbers, along with pack locations and wolf population size, over the Italian Alps in 2013, 2018, and 2023. I predicted 25 packs (95%CI – 19, 32) in 2013, 36 (95%CI – 23, 47) in 2018, and 49 (95%CI – 29, 68) in 2023. The South-Western Alps were the main source for wolves repopulating the Alps from 1999-2008. This main source area will likely be shifted to the Cozie Alps after 2008. In the next 15 years, the primary source for wolves repopulating the Alps will likely move to the north which could allow the full recolonization of the Eastern part of the Alps.

for Davide & Edo

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CHAPTER 1. EXTENDED INTRODUCTION

The wolf return in the Western Alps of Italy, France, and Switzerland

Wolves (*Canis lupus*) were deliberately extirpated throughout most of Western Europe during the 20th century, yet populations never went extinct in Spain, Portugal, and central Italy (Boitani and Ciucci 1993, Petrucci-Fonseca and Promberger 1993, Vila et al. 1993). However, even in these countries, wolf populations faced a sharp decline due to the hard ecological conditions characterized by the reduction of their natural prey species, deforestation, and human persecution (Promberger and Schroder 1993, Boitani 2003). Given these conditions, wolves have shown considerable ecological plasticity, surviving on both domestic and native prey sources in mountainous areas (Boitani 1982).

In Italy, wolves were widespread until the early 1900's when they were gradually extirpated in the Alps. The last wolves were killed in the Western Alps region during the 1920s, but wolves survived along the Apennines range of central Italy (Boitani and Ciucci 1993). Zimen and Boitani (1975) conducted the first wolf study in Italy, and documented that the Italian wolf population in 1973 was restricted to no more than 100 individuals in 2 fragmented subpopulations in southern and central Italy, inhabiting only about 5% of their original range in the Apennines Mountains (Fig. 1).

The Italian wolf population has no mitochondrial DNA (mtDNA) variability, and a private haplotype (W14) that allows researchers to easily distinguish it from any other wolf population (Randi et al. 2000). The observed mtDNA monomorphism is the likely outcome of random drift in the declining and isolated Italian population, which likely had a low effective population size during the last 100-150 years (Randi et al. 2000). Isolation of the Italian wolf population from the rest of Europe was apparently even longer than this; in fact, Lucchini et al. (2004) suggested the Italian population south of the Alps has been genetically isolated for thousands of generations. Hence, the Italian wolf population has recently been recognized as a valid subspecies (*Canis lupus italicus*) on both a morphological (Nowak 2003) and a genetic basis (Lucchini et al. 2004).

By the 1970s the importance of the wolf as part of a naturally functioning ecosystem came to be understood. The wolf was legally protected in Italy in 1971 (Boitani 1982), listed as an endangered and strictly protected species in Europe after the

Bern Convention in 1979, and after the Habitat Directive in 1992, and in the world after the International Union for Conservation of Nature Red list (IUCN 2001). Ecological conditions in Western Europe recently improved and both wild ungulate and wolf populations increased (Francisci and Guberti 1993). The range of wolves along the Apennines mountains increased over the years: in 1983 about 220 wolves were estimated in Italy (Boitani 1984), and recently about 600 wolves have been estimated over the Italian peninsula (Boitani 2003).

Wolves began naturally recolonizing the southwestern Alps of Italy and France at the beginning of 1990s (Poulle et al. 1995, Marucco 2001) through dispersal from the north Apennine wolf subpopulation (Fabbri et al. 2007) (Fig. 1). A moderate bottleneck occurred during the recolonization process, and gene flow between the Apennines and the Alps was moderate (corresponding to 1.25-2.50 wolves per generation) (Fabbri et al. 2007). Bottleneck simulations showed that a total of 8-16 effective founders explained the genetic diversity observed in the Alps (Fabbri et al. 2007). Therefore, the levels of genetic diversity in the current expanding alpine wolf population will depend on future successful migrants from the Apennines.

After 1995, wolf packs stabilized and increased in the Ligurian-Maritime Alps of Italy (Marucco 2001), and France (Poulle et al. 2000), and solitary individuals from the Italian wolf population appeared in Switzerland (Valiere et al. 2003). When these semi-isolated packs and individuals appeared progressively further from source wolf populations, questions arose regarding wolf numbers, actual distribution, and the origin of the animals. Livestock depredations increased in the Italian Alps with more than 900 sheep killed between 1999 and 2005 (Tropini et al. 2005), and about 2800 sheep killed in France only in 2004 (Duchamp 2005). In these past decades alpine shepherds have lost their tradition of coexistence with large predators, and lacked knowledge of preventive measures.

The Piemonte Region of Italy, together with the European Community, implemented a long-term wolf conservation program and funded the "Progetto Lupo Regione Piemonte" in the Italian Alps from 1999 through the present to document and quantify the dynamics of the wolf recolonization process and implement preventive measures for livestock depredations to improve human-wolf coexistence. At the same time long-term projects were conducted in France by the Office National de la Chasse et de la Faune Sauvage (ONCFS) (Duchamp et al. 2002) and Switzerland by KORA (Weber 2008) to follow the wolf recolonization process over the Alps. This exceptional, extended period of funding allowed the study of the wolf recolonization process in the Alps and the beginning and strengthening of a strict collaboration between the French, Italian, and Switzerland wolf monitoring groups, which today constitutes the Wolf Alpine Group (WAG). The WAG is a technical group comprised of the research institutions of Italy (Regione Piemonte, Centro Grandi Carnivori, Progetto Lupo Regione Piemonte), France (ONCFS, Réseau loup), and Switzerland (KORA), and is in charge of the wolf monitoring in the Alpine area.

In 2006, the "*Protocollo di collaborazione italofranco-svizerra per la gestione del lupo nelle Alpi*" ("Italian-French-Swiss collaboration protocol for the wolf management in the Alps"), was signed by the French, Italian, and Swiss governmental authorities (i.e. Ministère de l'Ecologie et du Développement Durable, Ministero dell'Ambiente e della Tutela del Territorio, Office Fédéral de l'Environnement) to facilitate collaboration regarding wolf management in the Alps. The document recognized the wolf population in the Alps as a distinct geographic entity for the purpose of management. The Large Carnivore Initiative for Europe (LCIE) also recently recognized the Western Alps wolf population as a unique population segment (Linnell et al. 2007), connected demographically and genetically to the Italian wolf population in the Apennines, but different in ecological and socio-economic contexts and from a management perspective (Linnell et al. 2007) because it inhabits the alpine area within different countries (i.e., Italy, France, and Switzerland). Consequently, the Wolf Alpine Group was recently appointed the technical advising board for wolf management by French, Italian and Swiss governmental authorities. Therefore, in this framework, standardized methods to collect, process and analyze the data are needed to monitor the wolf recolonization process across countries that share this alpine population. This PhD dissertation fits into this framework.

Spatial population dynamics and individual-based modelling of wolves in the Alps with non-invasive methods: motivation and directions

The natural expansion of wolves in the Alps is a great challenge for conservation biologists and wildlife managers because our goal is to achieve a viable population of large carnivore, while minimizing the conflict that wolves might generate in an environment with high human densities and high levels of habitat loss and fragmentation. Human population density in Piemonte Region is 138 people/ km^2; comparatively, the human population density in Montana is 6 people/ km^2. Such a complex conservation issue requires an understanding of the spatial and temporal dynamics of the wolf population. How large a population of wolves in the Alps must exist to be viable and whether it is possible to allow any removal of wolves for damage control purposes on the French or Swiss side of the Alps is still controversial. Therefore, research programs were expected to provide a quantitative understanding of the genetic, demographic, and spatial aspects of this Alpine wolf population, which are fundamental to building an effective management strategy.

Reliable estimates of population parameters and assessment of trends are necessary for effective management and conservation actions. However, such parameters are often very hard to obtain for elusive, rare, wide-ranging, and cryptic species. Population size and trend, as well as characteristics of a species distribution are important indices used to define the conservation status of a species under Habitat Directive 92/43/CEE, as well as the Endangered Species Act and the International Union for Conservation of Nature (IUCN) Red list (IUCN 2001). Spatial distribution parameters, such as occupancy rates, or the rate of change in occupancy over time, are important estimates which define the expansion or decline of a species range, and the relative occupancy dynamics (i.e. the same amount of range can change shape and become more or less fragmented) (MacKenzie et al. 2006). Management decisions that support the natural recolonization of this alpine wolf population are therefore dependent on reliable estimates of population size over time and other population parameters, such as survival rates, or occupancy rates. However, large carnivores, such as wolves, are difficult to study because they are often wide-ranging and at low densities (Boitani 2003) and these parameters are hard to accurately estimate.

Fuller et al. (2003), in a overview of wolf population dynamics, specified that knowledge gaps where further research is needed involved the development and implementation of standardized, accurate, and cost-effective methods of assessing wolf abundance for monitoring wolf recovery, harvest, and control actions. Ciucci et al. (2007) stressed the importance of accurate estimates of wolf survival and mortality in Europe, fundamental for any rational approach to wolf management and conservation. Lovari et al. (2007) also cited the lack of such information both in Italy and Western Europe.

New opportunities for estimating population parameters have been created with the growing use of molecular identification of individuals (Taberlet et al. 1999, Mills et al. 2000, Creel et al. 2003). Populations can be examined without ever seeing or handling the animal by extracting unique DNA from hairs or fecal samples and assigning an individual a “molecular tag” based on its genotype for several highly polymorphic microsatellite loci (Kohn and Wayne 1997, Mills et al. 2000). This approach allows estimation of population parameters for many species that were previously difficult to study. For example, using feces and hairs, Settlage et al. (2008) estimated population size for American black bears (*Ursus americanus*); Banks et al. (2003) estimated the number of northern hairy-nosed wombats (*Lasiorhinus krefftii*); and Roman and Palumbi (2003) estimated historic population size for whales in the North Atlantic Ocean. Genetic markers could then be used to track individuals throughout their lives and genetic mark-recapture techniques are used to estimate population size, survival, and movement. However, a variety of potential pitfalls can cause underestimation (Mills et al. 2000) or overestimation of the population size (McKelvey and Schwartz 2004). Finally, modelling approaches are promising today, especially because increased computer speed has led to the rise of individual-based models that include spatial complexity and behavioral components (Lyndenmayer et al. 2001, Stephens et al. 2002). Spatially explicit models are natural candidates for modelling population viability in fragmented landscapes (Melbourne et al. 2004). However, the usefulness of spatially explicit models for population viability analysis has been questioned recently because of high parameter uncertainty (Ruckelshaus et al. 1997, Beissinger and Westphal 1998). Boyce (2000: 254) suggested that modelling should always be “considered as a way of thinking and structuring ideas rather than a technique." Models can be used to test assumptions, guide the collection of data, and improve the understanding of system dynamics (Dunning et al. 1995, Boyce 2000). Indeed, all models are wrong at some level, but many models are useful for framing data and explicitly understanding how everything fits together (Boyce 2000). In this framework, I combined field non-invasive techniques and modelling approaches to investigate the natural recolonization process of a wolf population in the Western Alps of Italy and France. I wanted to provide quantitative understanding of the genetic, demographic, and habitat selection aspects of the Alpine wolf population, which will be fundamental to building an effective management strategy for long-term wolf conservation in the Alps.

Overview of the Dissertation

The primary goal of my research is to gain a landscape and population level understanding of the wolf recolonization process merging the use of non-invasive genetic sampling, capture-mark-recapture (CMR) analysis, occupancy analysis, Geographic Information System (GIS), modelling, computer simulations, and statistical analyses With such a model, it becomes possible to predict future wolf population development in the Alps at the pack level, which has important conservation and management implications.

More specifically, I had 4 primary objectives:

1. Estimate abundance, apparent survival, and sex ratios of wolves through time using genetic CMR techniques.
2. Discuss the limitations and pitfalls of non-invasive CMR approaches to gain these estimates.
3. Estimate wolf occupancy parameters, distribution, and develop a wolf habitat suitability model for the Italian Alps range.
4. Develop a spatially explicit, individual-based model that incorporates information obtained in the above objectives for analyzing the natural expansion of wolves under current conditions and predict future wolf pack development in the Alps.

In Chapter 2, I present and use a new non-invasive approach for estimating wolf survival rates, population size, and trend based on genetic CMR techniques applied over large areas. I applied open-population models to long term fecal genotyping data to estimate survival rates and monitor the wolf population trend in the Western Alps over eight years (1999-2006). I designed a scat sampling strategy to minimize the effect of the marking behavior of wolves which could otherwise cause individual heterogeneity in recaptures. I implemented a new method to accurately estimate wolf abundance and survival on a large scale. This new, non-invasive CMR application produced the first wolf survival estimates in Italy and in the Alps, and the first precise estimates of wolf population size through time in the Alps, and could be used as a standardized method to monitor wolf population parameters over the entire alpine range.

However, the use of non-invasive datasets in a CMR framework is not without limitations. Therefore, in Chapter 3, I discussed unique situations which could occur with genetic recaptures which are not readily apparent to ecologists unfamiliar with genetic data. I highlighted the pitfalls and limitations of the genetic CMR approach, the importance of a well-structured study design based on the biology of the species, the role of ecologists and geneticists throughout the process, and the problem of error propagation.

In Chapter 4, I used multi-season occupancy models to estimate detection probabilities and occupancy rates of wolves using data on wolf signs (i.e. snow-tracks and scats) collected over 5 years in the Western Italian Alps. I designed a monitoring program based on multiple visits to sites during each season to detect wolf signs over the entire Piemonte Region, Italy. I addressed 3 objectives to evaluate wolf sign surveys as a technique to measure spatial patterns of wolves. First, I determined the most important variables which determine wolf occurrence in an area taking into account the detection probability of wolf sign. Second, I investigated wolf occupancy dynamics in the Alps area, explicitly modelling potential changes in the occupancy state of a site over time with colonization and extinction probabilities. Third, I developed a GIS wolf prediction map on the Italian Alpine range, based on occupancy modelling estimates, which will be useful for management, and fundamental for the development of the individual-based spatially explicit model described in Chapter 5.

Finally, in Chapter 5, I analyzed the complex spatial and temporal population dynamics in this fragmented landscapes using a spatially explicit, individual-based model (SE-IBM) (Turner et al. 1995, Letcher et al. 1998, Wiegand et al. 2004). This type of model is optimal for analyzing the impact of spatial process and landscape structure on population dynamics (Pulliam et al. 1992, Dunning et al. 1995), and for examining the effects of human-induced landscape change on carnivores (Carroll et al. 2003). I developed a SE-IBM based on demographic processes, social structure and behavioral aspects, and habitat selection of wolves. The model followed the fate of individual wolves and simulated reproduction, home range establishment, mortality, and movement in annual time steps with a demographic submodel that simulated life-history events. The model incorporated the map of habitat suitability I developed in Chapter 4 and population parameters and data obtained in Chapter 2, and relates demographics of the species explicitly to the Alps landscape in which these wolves live. The SE-IBM estimates, population size and number of packs, along with pack structure, formation, and spatial distribution as secondary predictions. This SE-IBM successfully followed and modeled the recolonization process, is a useful management tool for prioritizing conservation efforts and data needs, and can be improved as new information and data are obtained (i.e. it can be used in adaptive management). My approach to analyze and understand such complex large carnivore spatial and temporal population dynamics in fragmented landscapes using spatially explicit individual-based models (Pulliam et al. 1992, Dunning et al. 1995) based entirely on information collected through non-invasive approaches could be applied in analyzing dynamics of rare and endangered species in which the use of other standard methods precludes an understanding of the state of the population.

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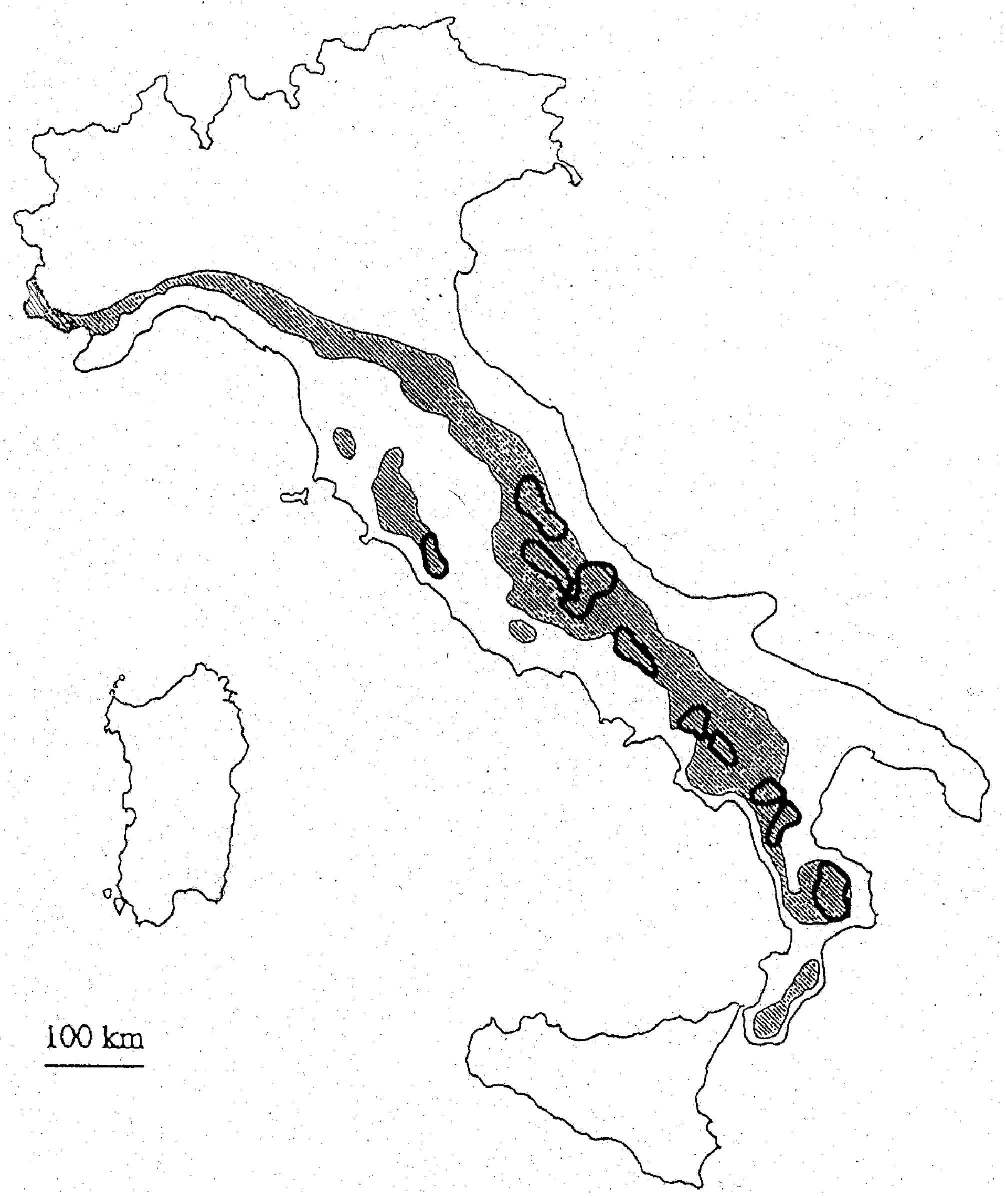

Fig. 1. Wolf distribution in Italy in 1975 (dark line, Zimen and Boitani 1975), and in 1998 (shaded area, Corsi et al. 1999).

CHAPTER 2. WOLF SURVIVAL AND POPULATION TREND USING NON-INVASIVE CMR TECHNIQUES IN THE WESTERN ALPS

ABSTRACT

The wolf naturally recolonized part of its former habitat in Western Europe. Effective management of this species relies on estimates of wolf population trend and survival rates. These estimates, key components for population models used to predict extinction probabilities and harvest scenarios, are lacking in Italy and Western Europe, and accurate and cost effective methods to assess these parameters have not been implemented. We used genetic capture-mark-recapture (CMR) techniques with long-term fecal genotyping data and applied open-population models to estimate survival rates and assess trend in abundance of wolves in the Western Alps over eight years (1999-2006). We designed a scat sampling strategy to minimize individual heterogeneity in recaptures, thus potentially biasing estimates. 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, but this constitutes a lower growth rate ($\lambda = 1.04 \pm 0.27$) than other recolonizing wolf populations. We found a positive trend in wolf abundance regardless of the method used, although snow-tracking yielded on average 36.2% (SD=13.6%) fewer wolves each season than CMR modelling. Young wolves, likely dispersers, which are characterized by low recapture probabilities, are taken into account in CMR estimates, which increases the overall population size estimate. We successfully implemented a new method to assess population trend and survival on a large-scale. These are the first wolf estimates obtained in Italy and in the Alps and, as such, will be important for guiding management decisions. Our approach can be widely applied to broader spatial and temporal scales for elusive and wide-ranging species in Europe and elsewhere.

INTRODUCTION

Reliable estimates of population parameters and assessment of trends are often necessary for effective management and conservation actions; however, such parameters are often very hard to obtain for elusive, rare, wide-ranging, and cryptic species. Population size and trend are primary factors that determine whether species are listed as endangered or threatened under the Habitat Directive 92/43/CEE, as well as the Endangered Species Act and the International Union for Conservation of Nature (IUCN) Red list (IUCN 2001). Estimation of population size and trend is also central to the management of many harvested wildlife species, and are especially important for taxa with low reproductive rates, which are most susceptible to over-harvest (Garshelis and Hristienko 2006).

Wolves recently recolonized the Western Alps through dispersal from the Apennines (Fabbri et al. 2007) after being extirpated throughout most of Western Europe during the 20th century. Management decisions that support the natural recolonization of this alpine wolf population are dependent on reliable estimates of population size over time and other population parameters, such as survival rates. However, large carnivores, such as wolves, are difficult to study because they are often wide-ranging and at low densities (Boitani 2003). Wolf monitoring programs often have relied on high levels of aerial radiotelemetry effort (Ballard et al. 1995, Hayes and Harestad 2000, Fuller et al. 2003). Other techniques, such as field observations, trapper questionnaires, radiotelemetry conducted on relatively few individuals, extrapolations of habitat suitability to estimate abundance, and ground-based telemetry studies, can approximate or index the regional population but lack the advantages of formal estimates of population size (Fuller and Sampson 1988, Mech et al. 1988, Fuller et al.1992, Boitani 2003), and are poorly suited to assess population trends (Garshelis and Hristienko 2006).

The wolf is particularly elusive in Western Europe; thus a telemetry based monitoring approach was both economically and logistically infeasible. Typical non-invasive monitoring based on howling surveys and snow-tracking (e.g., Ciucci and Boitani 1999) do not allow the estimation of these important population parameters. Attempts to estimate wolf mortality in Italy (Lovari et al. 2007) stressed the need for unbiased estimates of survival and other population parameters to produce information for managing populations on a more rational basis (Ciucci et al. 2007, Linnell et al. 2007). Methods have been developed to extract DNA and determine microsatellite genotypes from scats (Taberlet et al. 1999). Genetic markers can then be used to track individuals throughout their lives. Capture-mark-recapture (CMR) methods to estimate population size and survival can be applied to genetic data if individuals are sampled often enough to estimate recapture probabilities (Nichols 1992).

We used genetic CMR techniques to monitor wolf population size over eight years (1999-2006) and estimate survival rates in the Western Alps. Our approach relied on a sampling design based on large-scale snowtrack monitoring to collect wolf scats and aimed at minimizing the effects of differential marking behavior of wolves to minimize individual heterogeneity in recaptures. Additionally, we investigated factors that could increase the probability of creating a new individual through genotyping errors. Finally, we applied open population models to long-term fecal genotyping data to estimate survival rates and wolf population trend in the Alps.

MATERIAL AND METHODS

Study area

The study area, located in the Western Alps, encompasses the Ligurian, Maritime, and Cozie Alps. The area consists of about 4,000 km^2 and includes parts of Italy and France (Fig. 1). Each country consists of a mosaic of land management classes; however, part of the territory is protected in the Alta Valle Pesio and Tanaro Natural Park (67.7 km^2), the Alpi Marittime Natural Park (278.3 km^2), and the Mercantour National Park (685 km^2) (Fig. 1). 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 (e.g., *Abies alba, Larix decidua* and *Fagus sylvatica)* are prevalent, covering about 50% of the area; the remainder of the study area consists of alpine meadows and shrub land/rock areas. The snow-season generally lasts from October-November through April-May.

Sampling design for scat collection

Scat collection

We collected scats along wolf tracks using snow-tracking techniques. A few days after a snowfall, we conducted ski- or snowshoe-based surveys with crews of 4-20 people to search for wolf tracks by traveling transects that systematically monitored the entire study area. When we found wolf tracks, we followed them, first in the opposite direction of wolf travel to avoid disturbing the wolves, and then, on subsequent days, we continued by following wolf routes in both directions. In this way, we traveled a continuous wolf track on different days and defined this as a "snow-tracking session". We collected every scat along the wolf track, and estimated each pack size as the maximum number of wolves traveling together that winter, as recommended by Mech (1982). We also opportunistically collected fresh scats when encountered during other field activities. We stored each wolf scat at -30°C until transferred to test tubes containing either 95% ethanol or silica gel. We used sterilized material in transferring scats into test tubes to minimize contamination.

Sampling sessions

We covered all transects 1 to 7 times during each of two winter sampling sessions: an early winter session (EW) from October to January and a late winter session (LW) from February to May. We defined the timeframe of the two winter sessions based on the biology of wolves. In the first part of the winter (EW) the pups, born in the summer, join the pack in their daily travel movements and the pack is large and coherent. In the second part of the winter (LW) the alpha pair separate for reproduction and the female is gestating, the pack is often split, and solitary individuals are relatively common (Mech and Boitani 2003). We non-randomly selected a sub-sample of scats for the genetic analysis due to funding limitations. We prioritized fresh scats for the genetic analysis to optimize laboratory success (Lucchini et al. 2002), groups of scats found along the same snow-tracking session to increase the probability of characterizing each individual in the pack, and single scats found in territories where the presence of a wolf pack was not documented.

Measures of effort

We measured effort in two ways, defined as:

1) Field Effort, or the sampling effort per area (Fig. 1), was expressed as: Average number of surveys per session * Average number of people per survey in each area. A categorical variable was used for CMR analysis: low effort was defined when this value was < 60, and high effort was defined when this value was > 60.

2) Lab Effort, or the final number of scats successfully analyzed and used in the CMR analysis per session, reduced from the original collected scat sample because of funding limitations and laboratory performance, and we measured it by: Analysis rate (i.e., the percentage of the collected samples that were analyzed in the lab) * Success rate (i.e., the percentage of the samples analyzed in the lab that gave positive multilocus genotypes) for each session. These values were standardized by subtracting the mean and dividing by the standard deviation.

Analysis of marking behavior

We documented the marking behavior 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 (<40m) to a junction, pass, ridge, or ecotone, represent a strong marking behavior of the wolf which deposited the scat (Vila et al. 1994). Conversely, scats off trail, far away (>40m) from junctions, passes, ridges, and ecotones, and not on a higher strata, represent an absence of marking behavior of the wolf. We assigned a unique marking behavior value (UMB) to each scat. Scats received 1 point for each documented marking category. Points were summed to give the UMB value to each scat. For each wolf, higher UMB values of its deposited scats correspond to a more intense marking behavior, and therefore scats which are more likely to be detected (e.g., scats on trail/ road versus 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 test if we could collect these harder to detect scats using snow-tracking sampling. 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 a χ^2 test of independence.

Genetic laboratory analysis

DNA extractions, microsatellite and mtDNA analysis, sex determination, and probability of identity

We extracted DNA from scat samples using a 1.5cm^2 piece of the outside crust and *QIAMP DNA Stool Minikit* (*QIAGEN Inc.*, Germany). Tissue samples from dead recovered wolves were also extracted using standard protocols (QIAGEN Inc.). Scat samples were extracted in a facility only used for non-invasive DNA to avoid potential contamination from other sample types. Following Waits et al. (2001), we calculated the theoretical probability that two random individuals ($P_{(ID)}$) and two full siblings ($P_{(ID)sib}$) have the same genotype, respectively, and we selected the loci which produced at least a $P_{(ID)sib} < 0.05$ (Mills et al. 2000). Six variable microsatellite loci (*CPH2, CPH5, FH2088, FH2096*, *FH2137* and *CO9.250*; Fredholm and Winterø 1995, Francisco et al. 1996, Ostrander et al. 1993) used previously in studies of Italian wolves (Lucchini et al. 2002) were amplified in all scat and tissue samples. A total of 10 microsatellites were analyzed for each scat of each unique genotype of one pack due to a documented inbreeding event in 2005, to improve the probability of identity of the pups of this inbred pack. For these samples, we therefore ran 4 more microsatellites (FH2054, FH2140, FH2161, Pez 17) (Francisco et al. 1996, Neff et al. 1999). Scats were amplified using a multi-tube polymerase chain reaction (PCR) approach (Taberlet et al. 1996). Our PCR reactions for tissues were conducted at volumes of 10 μl containing 50-100 ng purified genomic DNA, 10mM Tris-HCL (pH 8.3), 50mM KCL, 2.0mM $MgCl_2$, 0.2mM of each dNTP, 0.2 μM of each primer and 0.5 U *AmpliTaq* DNA polymerase (Applied Biosystems). We modified this protocol with scat samples by using 2.5μl of DNA preparation, along with 2 μg/ml BSA and 1 U *AmpliTaq Gold* DNA polymerase. The thermal profile for the PCR reaction was 94 ^{o}C for 5 minutes, followed by 30 cycles of: 94 ^{o}C for 30 sec, 56 ^{o}C for 30 sec, and 72 ^{o}C for 30 sec. PCR profiles for scat samples were similar, but all steps were increased to 1 minute, and 45 cycles. PCR products were visualized on a Li-Cor 4300 DNA Analyzer (Li-Cor Biosciences, Lincoln, Nebraska USA). The Italian wolf population is genetically distinguishable from domestic dogs and other European wolf populations using either mitochondrial (Randi et al. 2000) or nuclear DNA (Randi and Lucchini 2002). Thus, each sample that had genotypes inconsistent with belonging to a wolf population was additionally analyzed at mtDNA to recheck species identification. Once the sample was confirmed to be from a wolf, we proceeded with sex determination using the ZFX/ZFY genes. Samples were first amplified using primers P15EZ and P23EZ previously used in wolves (Lucchini et al. 2002) followed by a nested PCR using primers ZFKF_203L and ZFKF_195H (Ortega et al. 2004). Nested PCR products were subsequently digested using restriction enzyme Taq$^{\alpha}$I to identify males and females (Garcia-Muro et al. 1997, Ortega et al. 2004). Finally, for each confirmed genotype, we ran 4 more microsatellites (FH2054, FH2140, FH2161, Pez 17) (Francisco et al. 1996, Neff et al. 1999).

Error checking protocol to reduce genotyping errors

We minimized the probability of creating a new individual through genotyping errors using multiple approaches based on protocols by Taberlet et al. (1996), Paetkau (2003), McKelvey and Schwartz (2004, 2005), Kalinowski et al. (2006), and on the use of independent field information. To detect errors, first 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 failed completely, or were inconsistent between the three amplifications, were re-extracted and re-amplified. Second, we grouped identical and near identical genotypes using the program Gimlet (Valiere 2002) to find samples that may have been erroneous using the logic that samples without matching genotypes were the most likely ones to contain errors (Prugh et al. 2005), as well as all the samples that differed only by one or two mismatches (1 or 2-MM pairs) (Patkeau 2003). Every such sample was systematically reanalyzed. Third, every confirmed genetic result was then compared to the snow-tracking information of the track followed, the direct observation data, the dead recovery data, or the radiotelemetry data for independent field confirmation. We conducted additional extractions and amplifications for all the samples which produced data incongruent with field information, until the dataset was satisfactory. Fourth, we then ran 4 more microsatellites (FH2054, FH2140, FH2161, Pez 17) (Francisco et al. 1996, Neff et al. 1999) for each unique genotype identified to improve estimates of error rates making the dataset more amenable for conducting the EB test and the DCH test (McKelvey and Schwartz 2004) using program DROPOUT (McKelvey and Scwhartz

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 error mode was 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, using a maximum likelihood method, for the presence of null alleles at each locus, as indicated by deficiency of heterozygotes relative to Hardy-Weinberg expectations (Rousset and Raymond 1995).

Parental relationships and age determination

We used a combination of relatedness analysis using the software ML-RELATE (Kalinowski et al. 2006), exclusion considerations, and field observations to estimate relationships between wolves. We defined pack membership for each genotype for every year, considering snow-tracking sessions and other field information. 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. Within each pack and each year, we evaluated the range of relationships available in ML-RELATE for the genotypes detected – unrelated, half-sib, full-sib, and parent-offspring – and identified the relationship with the highest likelihood (Wagner, Creel & Kalinowski 2006). Using this combination of analysis, 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 in one of these categories, the wolf status was considered unknown.

We then defined 3 age classes: adults (wolves > 1 year old, which were parents that year, or all the individuals present in the study area the year before); pups (wolves < 1 year, which were the documented offspring of the reproductive couple for that year); or unknown (wolves of which we do not know the starting age).

CMR estimation of population parameters

Each fresh wolf scat, collected and genotyped successfully, was considered a "capture or recapture" of the individual genotyped on the day of collection. We constructed a capture history for each wolf by recording whether it was captured or not in each sampling session. Only scat samples were counted as captures (dead recoveries were not included). 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. Apparent survival is the probability that an individual is alive in the study area. Following recommendations by Lebreton et al. (1992), we first 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 (Pradel et al. 1997) and trap-dependence (trap-happiness or trap shyness; Pradel 1993), which are particularly sensitive to heterogeneity in recaptures and independence of individuals. We defined a set of 20 biologically plausible models. We defined a set of 20 biologically plausible models, where survival probabilities were modeled to examine variation over seasons (summer and winter), age (young, adults, or unknown), and sex; and recapture probabilities were modeled as a function of years or seasons (EW and LW), to examine the importance of different general conditions of snow and weather within years/seasons, and age, sex, and effort (as measured by Lab and Field Effort). Lab 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 and Anderson 2002). We model averaged the best models with $AIC_c < 2$ to obtain averaged parameter estimates and standard errors (White and Burnham 1999). We estimated standard errors of the annual survival rates using the Delta Method (Cooch and White 2006). 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 x_1 (Burnham and Anderson 2002).

We used the estimates of recapture rates from the best CJS model to derive estimates of population size using a Horvitz-Thompson-type estimator ($\hat{N}_t$) (e.g. McDonald and Amstrup 2001):

$$\hat{N}_t = \sum_{s=1}^{n} \frac{I_{st}}{\hat{p}_{st}} \qquad \text{(eqn 1)}$$

where $\hat{N}_t$ is the estimated population size at time t, I_{st} is 1 if animal s was captured during time t and 0 if it was not, and $\hat{p}_{st}$ is the maximum likelihood estimate of the recapture rate of animal s at time t. The approximate variance of $\hat{N}_t$ is:

$$\text{var}\left(\hat{N}_t\right) = \sum_{s=1}^{n} \left(\frac{I_{st}\left(1-\hat{p}_{st}\right)}{\hat{p}_{st}^{2}} + \frac{I_{st}\sigma_{pst}^{2}}{\hat{p}_{st}^{3}} + \frac{I_{st}\left(1-\hat{p}_{st}\right)\sigma_{pst}^{2}}{\hat{p}_{st}^{4}} \right) \qquad \text{(eqn 2)}$$

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 of population size estimates, using a bootstrap approach with 1000 replicates using a working version of program M-SURGE kindly made available by R. Choquet. In turn, we compared the per season snow-tracking estimates of population size, the per season counts of simultaneous genotypes, the explicit CMR estimates of population size, and the bootstrap CMR estimates. We evaluated the finite rate of increase (λ) from late winter to late winter as the ratio of the population size during the next late winter to the population size for the current late winter.

RESULTS

Scat sampling

The total length of transects to search for wolf tracks and scats was 1846 km. We conducted on average 28 surveys/season with an average 3.5 people/survey; the average number of surveys/season/area ranged from 5 to 60, the average number of people/survey/area ranged from 2 to 5, to describe areas of lower and higher Field Effort (Fig. 1). Transects were covered to search 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 (7 early and 7 late winter sessions) (Fig. 1). We genotyped 1399 scats for a 41.4% analysis rate. We identified 87 unique individual genotypes from the 739 scats that provided successful results (a 52.8% lab success rate, as defined above). Analysis rates per sampling session ranged from 12.9% to 79.4% due to variation in scats and resource availability; lab success rates ranged from 15.4% to 77.8% due to lab performance and scat storing modes. The measure of Lab Effort varied between sampling sessions from -1.21 to 2.23. The number of recaptures (i.e., scats) per individual ranged from 1 to 56; 31.0% of the individuals were recaptured only once (Fig. 2a). We pooled the recaptures in each session, and considering the sampling sessions, 43.7% of the individuals were recaptured only once (Fig. 2b). All tissue samples from wolves that were found dead in the study period (*n*= 9) matched the genotype of ≥ 1 scat sample, as did all dispersing wolves that were occasionally sampled or found dead outside the study area in North-eastern Italy, Switzerland or Germany, (*n*= 6). Hence, the probability of capturing a wolf present in the study area during scat collection was high.

Analysis of marking behavior

We documented the marking behavior 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 total number of scats, 26.6% were from young wolves. However, young wolves comprised 54.0% of the individuals sampled, which indicates that young wolves were re-sampled fewer times than adults. The collection of a scat from a young wolf (or an adult one) was dependent on the location of the scat ($\chi^2 = 29.4$, df = 1, P<<0.001) (Fig. 3a) and on the UMB of the scat ($\chi^2 = 20.3$, df = 5, P=0.001) (Fig. 3b). In particular, scats from young wolves were more often found off-trail (54.6%) and in areas of a UMB of 0 (24.6%) than those from adult wolves, (respectively 28.1% off-trail and 12.8% of a UMB of 0) (Fig. 3a and 3b). 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 of young wolves. However, we avoided this bias because we collected scats along wolf tracks.

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 5 loci. The theoretical $P_{\text{ID-sib}}$ was

1.50e-02 with 6 loci. The probability of identity for siblings using the 10 loci was 7.845e-04 ($P_{\text{ID-sib}}$).

After conducting multiple error-checking approaches, we eliminated 46.3% of the initially estimated number of individuals obtained after the multitube approach. Ultimately we obtained 87 high quality genotypes. Using multiple error-checking approaches the average number of replicates conducted per sample per locus was 4.3 (SD= 1.6, range= 3-19), where the highest number of replicates were conducted on inconsistent samples. 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 the remainder 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, likely due to the presence of a partial null allele in locus *cph2*. DCH tests confirmed the removal of errors. After error removal, the tests for the presence of null alleles using ML-RELATE indicated no heterozygosity deficiency (H-W tests: $0.2 < \text{P-values} < 0.9$ for the 10 loci), or null alleles in any locus ($p_n < 0.0001$), 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 7 wolf packs in the area, 3 solitary wolves with a permanent territory, and 18 dispersers of which 6 dispersed outside the study area and 12 within the study area. For 5 packs we were able to reconstruct detailed relationships and identified the reproductive pair every year. When we examined the first time sampled for each individual, 54.0% of the individuals were young wolves (they were young only for the first year of sampling, than transitioned to adults), 19.6% were adults (adults sampled the first time as such), and the rest were of unknown age.

Wolf population trend and survival

The overall goodness of fit test was not significant ($\chi^2 = 8.911$, P = 0.998) and showed no sign of transience (z = 0.595, two-sided test, P = 0.552) nor of trap-dependence (z = - 0.672, two-sided test, P = 0.501), suggesting that the fully parameterized CJS model fit the data well. In particular, heterogeneity among individuals in detection probability was negligible. Because of the presence of young individuals in the population, with an expected lower survival, age-effects were nevertheless considered in the set of biologically plausible models. We then examined reduced-parameter models: the 3 best models included age and season as additive effects, affecting apparent survival (Φ) and the 2 measures of effort (Field and Lab) as main effects with an 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 considered. In all models, age was best explained if young and unknown, 2 of the categories for age, were considered equal. The sum of the Akaike weights for the pair of covariates age and season was 1.00, which indicates the high importance of these two variables in explaining the probability of survival. Young wolves had lower annual apparent survival rates (0.24 ± 0.06) than adult wolves (0.82 ± 0.04) (Fig. 4). Survival rates were lower in the summer than in the winter both for young and adults (Fig. 4). Recapture rates were best explained by Field Effort (Table 1). The sum of the Akaike weights for the covariate Field Effort was 1.00, indicating the high importance of this variable in explaining the probability of recapture; the weight for Lab Effort was 0.57, indicating a lower importance (Table 1). Sex had a minimal effect on survival and recapture rates, and was, therefore, absent from the top 15 models (Table 1). Averaged estimates of recapture rates varied from 0.28 ± 0.12 to 0.77 ± 0.07 depending on Field and Lab Effort (Table 2).

Estimates of population size derived from the Horvist-Thompson estimator suggested an increase in population size from 1999 to 2005, and a decrease in population in the last winter 2005-2006, with a general tendency to higher abundance estimates during early winter (Fig. 5). The log mean of the back-transformed estimates using bootstrapping followed the same trend (Fig. 5). The two types of estimates of log population size were as expected highly correlated ($r=0.9416$, $P<0.001$) (Fig. 5 and 6). Transforming the estimated population size to logarithms was sufficient to provide a more stable estimator, and had the further advantage of stabilizing the variance (Seber 1982), as well seen in comparing graphs in Fig. 6 from left to right for the highest values. Similar results were obtained by discarding (for each season) all estimates that were higher than 500 from the calculation of the mean bootstrap estimate (i.e. constrained mean in Fig. 6). 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 ML estimates and their associated standard errors estimates were by definition symmetrical. The expected coverage of the bootstrap confidence intervals was thus expected to be more reliable (i.e. to encompass with a coverage closer to the expected one, the unknown true value).

We detected a positive trend in wolf abundance regardless of the 5 methods used (Fig. 5), even though we estimated on average 36.2% (SD=13-6%) fewer wolves each season with snow-tracking data as we did by CMR modelling (Fig. 5). The number of packs detected show a trend similar to the CMR estimates but with less variation between the early and late part of the winter (Fig. 5). The averaged late winter to late winter finite rate of increase (λ) of the population was 1.04 ± 0.28. The high variation is given by the last year estimate, characterized by a decrease in the population size.

DISCUSSION

Wolf population trend and survival in the Western Alps

The wolf population increased over the 7 years of our study, as did the number of packs observed. All methods used supported this trend, though we estimated fewer wolves every season with snow-tracking data than we did by CMR modelling. Packs are the main social units of a wolf population, but the population is composed of lone wolves too, which are temporarily independent as they disperse from packs to either start their own packs or join existing packs (Fuller et al. 2003). Genetic CMR modelling emphasizes the role of these individuals which are counted in the genotype estimate but are characterized by low recapture rates, which indirectly increase the overall population size estimate. These dispersers are likely not detected through snow-tracking data. Populations of wolves that are colonizing an area can be expected to exhibit a variety of dispersal patterns, including long-distance movements (Pletscher et al. 1997), and these individuals are rarely recaptured unless they stabilize and form a new pack. Genetic CMR population size estimates should be corroborated with estimates of pack numbers as well as by dispersal patterns and survival rates, which is an estimate more robust to heterogeneity in recaptures (Lebreton *et al.* 1992), to correctly interpret population trends and identify the proportion of solitary wolves. This is especially true in newly recolonized areas, such as the Alps. In our study, the majority of the population is composed of young wolves, which is typical of wolf recolonization in areas with plentiful suitable habitat. Here, newly established packs can be made up of only a pair of wolves, so newborn pups form a large part of the population in such areas (Fuller et al. 2003). We detected a general tendency to higher abundance estimates during early winter, as expected based on the life cycle of wolves if immigration is low; hence, if we are to give only one population size estimate per year, the late winter estimate is likely more conservative. Similar results have been found in other wolf studies in North America (Mech 1977, Fuller et al. 2003).

Survival rates of young in the Alps were lower then in similar recolonizing situations elsewhere (e.g., Pletscher et al. 1997), and this likely explains the lower lambda compared to other studies (e.g., Hayes and Harestad 2000). During winter 2005-2006, the wolf population suddenly decreased, perhaps due to poaching (we documented illegal poisoning of two radio-collared wolves). Wolf populations can still be threatened by illegal killing or other stochastic sources of annual mortality, despite an overall positive trend in abundance. Apparent survival rates were best explained by age and season. Young had much lower survival rates than adults, and summer survival was lower than winter survival. In particular for young, the winter survival could be considered very close to true survival, because dispersal is very unlikely in the first 6 months of life (Gese and Mech 1991, Boyd and Pletscher 1999), whereas survival in the summer must be considered apparent survival, because it is impossible to distinguish between dispersal outside the study area and mortality. High turn-over of individuals within packs were documented for young, whereas the adults and the alpha pairs maintained their position for several years. Experience likely enabled them to survive in an ecosystem with high human presence. Survival of females was similar to that of males, as well as recapture rates, as has been reported elsewhere (e.g., Fuller et al. 2003).

Recommendations for non-invasive CMR monitoring studies based on scat collection

Genetic CMR techniques are a promising tool to estimate population parameters and monitor populations through time (Schwartz, Luikart & Waples 2007) for a variety of species and locations. However, limitations and specific sources of error are present using a non-invasive dataset in a CMR framework (Creel *et al.* 2003). The presence of capture heterogeneity is one of the most difficult problems facing estimation of animal abundance using CMR approaches (Pledger and Efford 1998, Lukacs and Burnham 2005). With wolves, problems of capture heterogeneity can arise during scat sampling due to differing behavior of individuals leading to disparate probabilities of finding their scats. Strong marking behaviour (e.g., at prominent sites or on trails) of dominant canids can increase their capture probabilities. In our study young wolves were more likely to be sampled with scats collected off or away from human trails, not 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 our sampling design based on collection of scats along wolf tracks increased the probability of characterizing each individual in the area, especially young and wondering wolves, thus minimizing the effects of behavioural differences in individual marking and allowing every wolf to be sampled despite their social status or other factors. The sampling of scats during snow-tracking sessions, together with the high effort to remove genotyping errors, produced a dataset which met the assumptions of homogeneity in recapture rates which is fundamental for CMR modelling. Moreover, the best models indicated that age was not an important covariate in explaining recapture rates, which means that we could sample young and adult in the same manner through sampling via snow-tracking, despite strong differences in their marking behaviour. 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.

Recapture rates in our study were strictly dependent on Lab Effort, and Field Effort, rather than the age of wolves, time, or season. Field Effort, which varied in our study due to logistics, was the most important covariate. Therefore, to minimize standard errors around population estimates in the future, it will be important to homogenize Field Effort between areas. Multiple recaptures of one individual within a sampling session is useless because these captures collapse together into a single detection. Therefore, considering the minimum lab success rate per season of 15% (i.e., full genotype one out of 7 scats), and the snow-track sampling protocol that detected half of the wolves present compared to CMR modelling (i.e. *2), we estimated that we will need to collect a minimum of ((7 * number of wolves present in the area from snow-tracking estimates) *2) scats to fully sample an area. This strategy will provide a wolf a reasonable chance of being detected in every session in every area, if the wolf is present. Using this rule of thumb will allow investigators to plan an annual budget for scat analysis based on a desired level of precision.

The probability of detection (i.e., the 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 a good indirect estimate of absence of genotyping errors in the dataset. We suggest acknowledging that genotyping errors might not be completely eliminated from any single lab protocol, and then working with a multiple approach to have the most reliable results for subsequent CMR analysis. We suggest that the best check on laboratory errors is through the use of independent field data (e.g., snow-tracking or radio-tracking information, dead recoveries, etc.), and the foresight to preserve some of the laboratory budget to re-examine any samples which produce results incongruous with field information. Partial null alleles, which are characterized by an allele with a mutation that amplifies weakly, have been often ignored as a source of genotyping errors (Wagner et al. 2006), especially in non-invasive population studies, where few have acknowledged and investigated this problem (e.g., Paetkau and Strobeck 1995). In non-invasive genetic CMR studies, partial null alleles are hard to detect because they produce systematic errors, and therefore create consistent false genotypes, which can be recaptured multiple times. In our study, we detected the presence of a partial null allele in locus *cph2*, which occasionally amplified. This type of error is very hard to notice without independent field data to check for the genetic dataset, and it should be regularly checked during lab analysis because it can have implications for both abundance and parentage.

A strict sampling design for scat collection is often harder to implement than the hair-snag sampling often conducted on ursids (e.g., Boulanger et al. 2004, Kendall and McKelvey 2008); therefore, open CMR models, like the CJS we used, should be implemented because they are best applied in a multi-year study to document population trends, are more flexible because they do not assume a closed population and a strict sampling design, are well suited for non-invasive samples and 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 a 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 and Manly 1985).

Management and conservation implications

Fuller et al. (2003) identified knowledge gaps for future research efforts including the development and implementation of standardized, accurate, and cost-effective methods of assessing wolf abundance to monitor wolf recovery, harvest, and control actions. Ciucci et al. (2007) stressed the importance of accurate estimates of wolf survival in Europe, fundamental for any rational approach to wolf management and conservation, and Lovari et al. (2007) noted the lack of such information both in Italy and Europe. We successfully implemented a new method for assessing wolf abundance and survival on a large-scale in a newly recolonized area in the Alps. This new, non-invasive

CMR application used on a large-scale project, produced the first wolf survival estimates in Italy and in the Alps, and the first precise estimates of wolf population size through time in the Alps.

The wolf population in the Alps has been identified as a unique population segment (Linnell et al. 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 and from a management perspective (Linnell et al. 2007) because it inhabits the alpine area within different countries (i.e., Italy, France, and Switzerland). Furthermore, it is a population that is currently recolonizing a considerable amount of unoccupied potential wolf range. The population trends and survival rate we estimated are necessary to guide important management decisions and understand the dynamics of this new alpine wolf population. PVA models have provided a means to examine the relative contributions of competing factors on rates of population change, giving important insights on how to manage the processes threatening species of conservation concerns (Brook et al. 2002). Despite this advance, most PVA models should rely on precise life history data from the population of interest (Ellner et al. 2002); often, however, researchers are forced to make assumptions when such data are missing (Bradshaw et al. 2007). 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 wolf population modelling analyses; however, true survival probabilities are still a missing piece of information.

Genetic CMR techniques are a promising tool to estimate population parameters and monitor populations through time (Schwartz et al. 2007) for a variety of species and areas. However, limitations and specific sources of error are present using a non-invasive dataset in a CMR framework (Creel et al. 2003). Improving the sampling design, developing species-specific protocols for specific objectives, improving lab protocols, and interpreting genetic results with field data, will improve CMR estimates. In interpretation of population trends, it is important to estimate additional parameters such as survival, which is more robust to the partial failure of assumptions like heterogeneity of individuals in captures (Lebreton et al. 1992). Wolf recovery appears to be continuing throughout the Alps, therefore, the approach described here will be fundamental in monitoring the population in the future across a broader area.

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Table 1. Top 15 Cormack-Jolly Seber models run in MSURGE to calculate maximum likelihood estimates of apparent survival rates (*Φ)* and recapture rates (*p)* of wolves in the Western Alps, 1999-2006. Models are ranked according to their AICc values. Models include the following covariates: sampling effort per area (Field Effort), the final genotyped sample size (Lab Effort), age, season, and time (t). We forced young and unknown to be equal, therefore "age" has 2 parameters, except where "3 age classes" is specified.

Model	AICc	ΔAICc	AICc weights	Model likelihood	No. of parameters
p (Field effort x Lab effort) *Φ* (age + season)	463.27	0	0.36	1.00	7
p (Field effort + Lab effort) *Φ* (age + season)	464.30	1.03	0.21	0.60	6
p (Field effort) *Φ* (age + season)	464.88	1.61	0.16	0.45	5
p (Field effort) *Φ* (age x season)	465.93	2.66	0.09	0.26	6
p (Field effort) *Φ* (3 age classes + season)	465.95	2.68	0.09	0.26	6
p (Field effort + t) *Φ* (age + season)	467.71	4.44	0.04	0.11	17
p (Field effort + age) *Φ* (age + season)	468.30	5.03	0.03	0.08	7
p (Field effort all areas) *Φ* (age + season)	469.59	6.32	0.02	0.04	11
p (Field effort) *Φ* (season effects 3 age classes)	474.42	11.15	0.00	0.00	7
p (Field effort) *Φ* (season effects young)	475.42	12.15	0.00	0.00	6
p (Field effort) *Φ* (age)	477.55	14.28	0.00	0.00	5
p (season) *Φ* (age + season)	479.77	16.5	0.00	0.00	5
p (Lab effort) *Φ* (age + season)	480.15	16.88	0.00	0.00	5
p (age) *Φ* (age + season)	481.35	18.08	0.00	0.00	6
p (age + season) *Φ* (age + season)	483.57	20.3	0.00	0.00	7

Table 2. Estimates and SE of apparent survival and recapture rates of the best model, and of the 3 best models averaged. These last maximum likelihood estimates were obtained averaging the 3 best Cormack-Jolly Seber models, as ranked by the AICc values.

Parameters	Covariates		Best model estimates	Best model SE	Averaged estimates	Averaged SE
Survival rates	Adults	Winter	0.98	0.01	0.98	0.02
		Summer	0.84	0.04	0.84	0.04
		Annual	0.82	0.04	0.82	0.04
	Young	Winter	0.78	0.09	0.78	0.09
		Summer	0.30	0.07	0.30	0.07
		Annual	0.24	0.06	0.24	0.06
Recapture rates	High Field Effort	Lab Effort in t_1	0.74	0.04	0.74	0.04
		Lab Effort in t_2	0.74	0.04	0.75	0.04
		Lab Effort in t_3	0.72	0.07	0.71	0.07
		Lab Effort in t_4	0.73	0.04	0.73	0.04
		Lab Effort in t_5	0.73	0.05	0.73	0.05
		Lab Effort in t_6	0.74	0.04	0.75	0.04
		Lab Effort in t_7	0.72	0.06	0.72	0.06
		Lab Effort in t_8	0.73	0.05	0.73	0.05
		Lab Effort in t_9	0.75	0.06	0.77	0.06
		Lab Effort in t_{10}	0.76	0.07	0.77	0.07
		Lab Effort in t_{11}	0.74	0.04	0.74	0.04
		Lab Effort in t_{12}	0.73	0.04	0.73	0.04
		Lab Effort in t_{13}	0.74	0.04	0.74	0.04
	Low Field Effort	Lab Effort in t_1	0.41	0.07	0.41	0.07
		Lab Effort in t_2	0.48	0.08	0.45	0.08
		Lab Effort in t_3	0.20	0.09	0.28	0.12
		Lab Effort in t_4	0.35	0.07	0.37	0.07
		Lab Effort in t_5	0.31	0.07	0.35	0.08
		Lab Effort in t_6	0.45	0.07	0.44	0.07
		Lab Effort in t_7	0.24	0.08	0.31	0.10
		Lab Effort in t_8	0.31	0.07	0.35	0.08
		Lab Effort in t_9	0.64	0.12	0.55	0.14
		Lab Effort in t_{10}	0.68	0.12	0.58	0.15
		Lab Effort in t_{11}	0.38	0.07	0.40	0.07
		Lab Effort in t_{12}	0.36	0.07	0.38	0.07
		Lab Effort in t_{13}	0.39	0.07	0.40	0.07

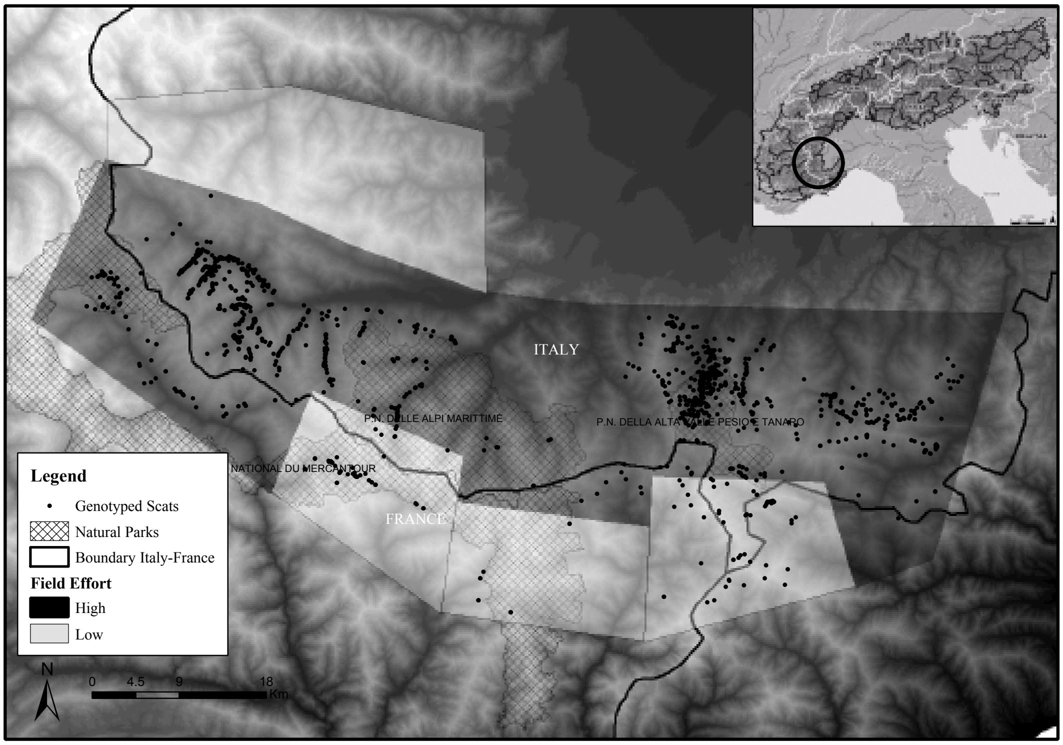
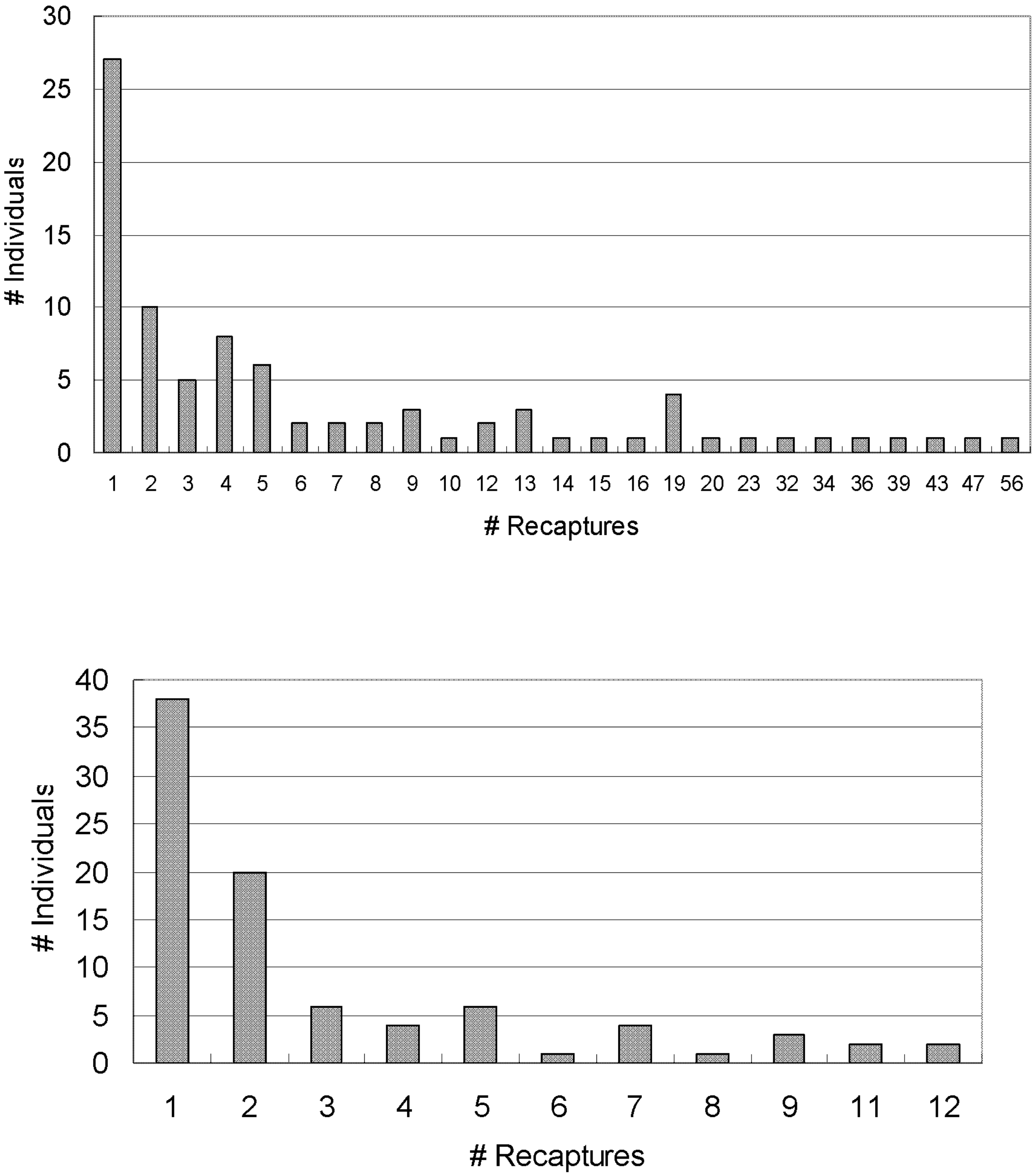


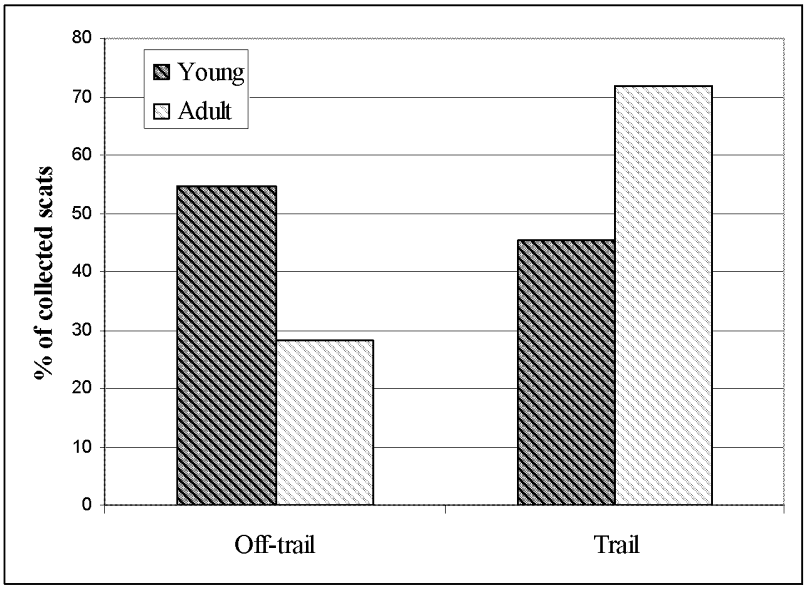
Fig. 1. Western Alps study area in Italy and France showing the genotyped scats locations, and the areas of low and high Field Effort.



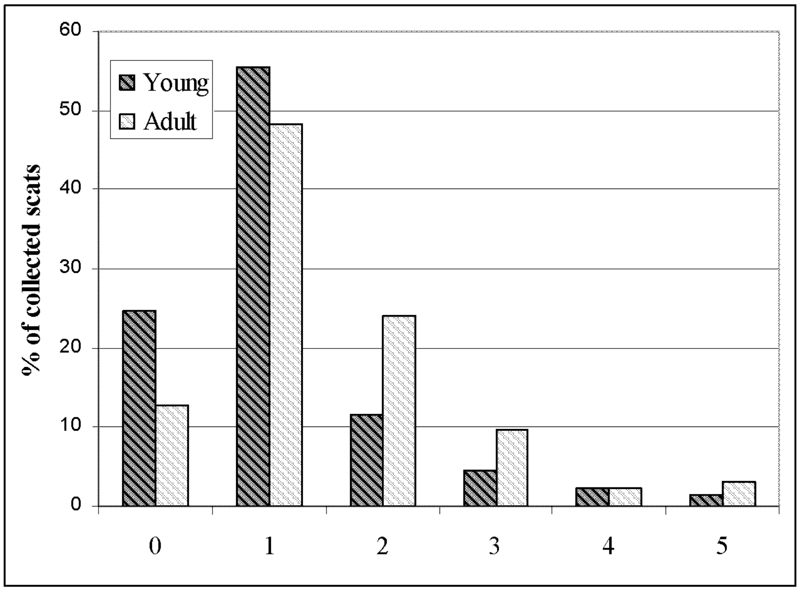
a)

b)

Fig. 2. Number of scats successfully genotyped per wolf (a), and per wolf per season (b) in the Western Alps study area, 1999-2006.



a)



b)

Fig. 3. Documented marking behavior of wolves related to each collected scat. Percentage of scats deposited by young (n=130) and (n=359) adult wolves off trail or on trail (a). Percentage of scats deposited by young (n=130) and (n=359) adult wolves with different levels of marking behavior, where a unique marking behavior value (UMB) was assigned to each scat (b). Higher values correspond to a higher marking behavior.

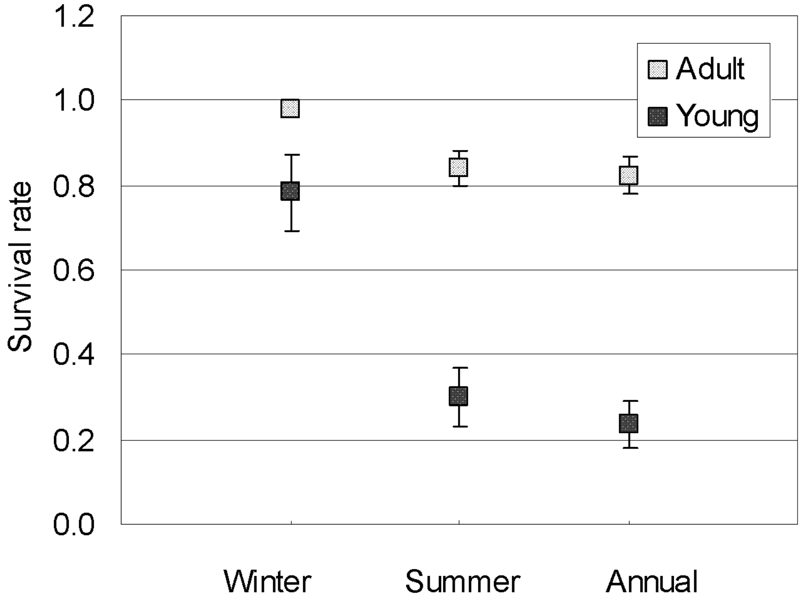


Fig. 4. Estimated winter, summer, and annual apparent survival rates for young and adult wolves in the Western Alps study area, 1999-2006. Error bars represents 95% confidence intervals. These maximum likelihood estimates were obtained by averaging the 3 best CJS models.

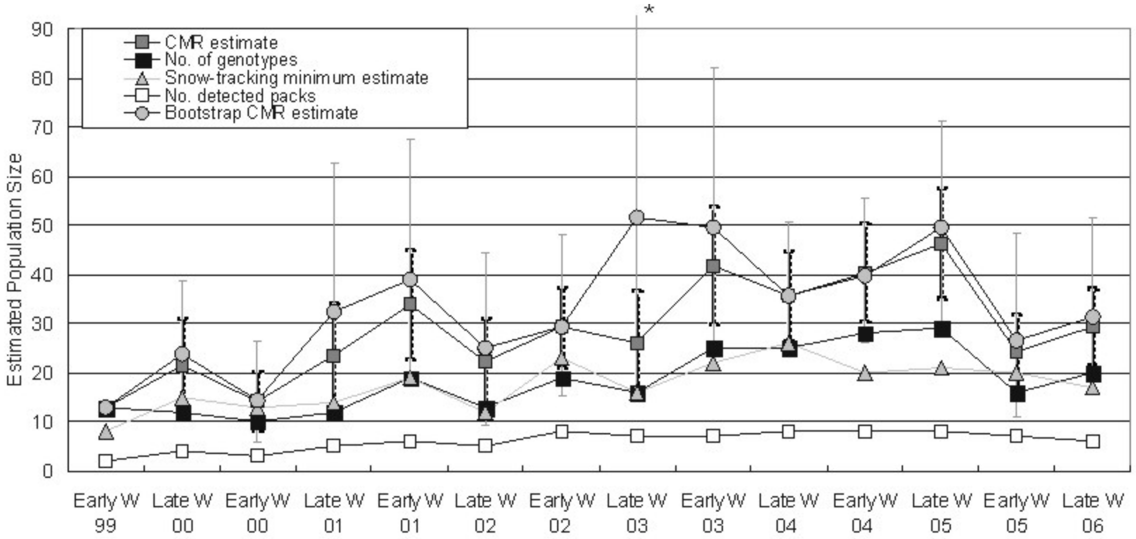


Fig. 5. Total estimated wolf population size in the Western Alps study area during each sampling interval, from the early winter 1999 (Early W 99) to the late winter 2006 (Late W 2006). Population size was estimated using the CMR estimates (±SE), the bootstrap CMR estimates (and 95%CI), the maximum number of genotypes detected per season, the snow-tracking estimate. * The top of the CI is 122.

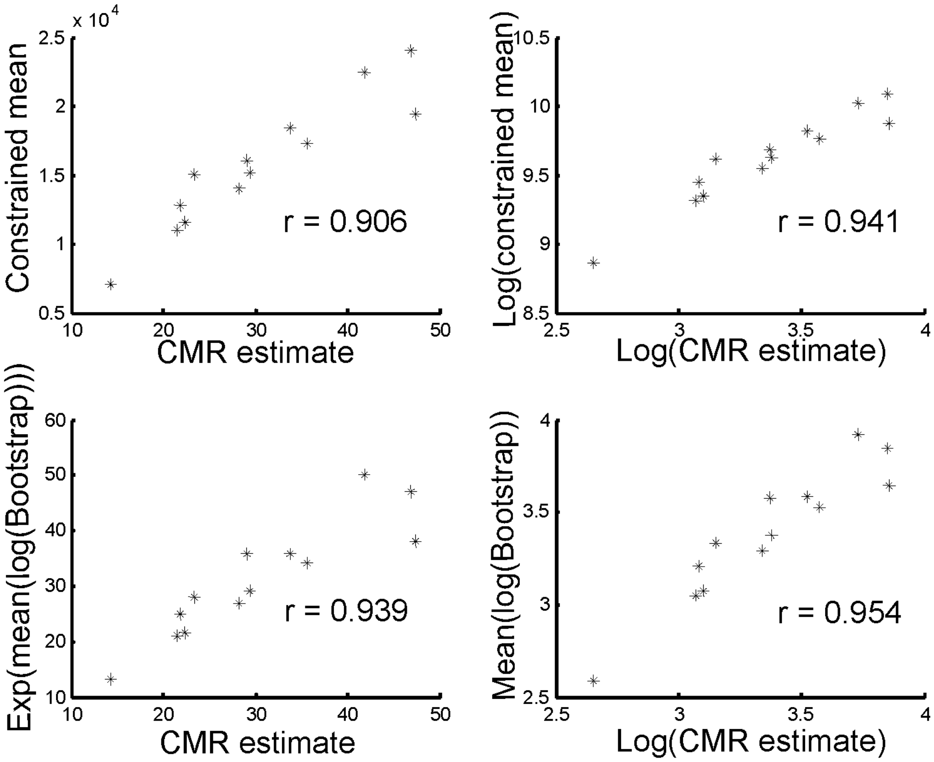


Fig. 6. Correlations between standard CMR population size estimates and bootstrap mean estimates of wolf population abundances in the Western Alps study area for each sampling interval, from the early winter 1999 to the late winter 2006. The constrained mean was obtained discarding (for each season) all estimates that were higher than 500 from the calculation of the mean bootstrap estimate.

CHAPTER 3. BRIDGING THE GAP BETWEEN MOLECULAR ECOLOGY AND FIELD BIOLOGY: A GUIDE TO EFFECTIVE INTEGRATION OF NON-INVASIVE GENETIC SAMPLING AND POPULATION PARAMETER ESTIMATION

ABSTRACT

Reliable population estimates are often necessary for effective management and conservation actions, and the use of genetic data for capture-mark-recapture (CMR) techniques has become an important tool to estimate population parameters for elusive species. Strong emphasis has been placed on the genetic analysis, or on the CMR applications; however, little attention has been paid to the simultaneous overview of the full non-invasive CMR approach, and the synergy gained by understanding both elements of the analysis. Here, we review the three main steps of the approach: designing the appropriate sampling scheme, conducting the genetic lab analysis, and applying the CMR analysis to the genetic results. We discuss the importance of the integration between these steps, the unique situations which occur with non-invasive studies, the role of ecologists and geneticists throughout the process, and the problem of error propagation. We highlight the importance of team collaboration and offer a series of recommendations to wildlife ecologists who are not familiar with this topic yet but may want to use this tool to monitor populations through time.

INTRODUCTION

Wildlife conservation and research has benefited from the field of molecular genetics (DeYoung and Brennan 2005, DeYoung and Honeycutt 2005, Allendorf and Luikart 2007). Our ability to delineate populations, understand dispersal patterns, detect hybridization, and count and monitor wildlife has improved through the synergy of traditional wildlife biology with molecular ecology. One area of wildlife research, in particular, that has benefited from molecular genetics is the estimation of animal abundance and other demographic parameters (e.g. Boulanger et al. 2004, Prugh et al. 2005, Kendall et al. 2009).

Historically, estimation of demographic parameters on species that are rare, elusive, difficult or expensive to capture has been limited due to small sample sizes. Advances in molecular genetics allows individual identification from the collection of non-invasive samples (hair, feces, etc.), often eliminating the need to capture or handle an animal to uniquely mark it (Taberlet et al. 1997, Kohn et al. 1999, Lucchini et al. 2002). Thus, "molecular tags" can now be used to track individuals throughout their lives and Capture Mark Recapture (CMR) methods can be applied to genetic data if individuals are sampled sufficiently to estimate recapture probabilities (Nichols 1992).

We advocate that wildlife biologists should have a solid understanding of the basics of genetic techniques to most effectively use genetic results to answer ecological questions. At the same time, geneticists should fully recognize the management and conservation implications of biological work, where data uncertainty, biases, and standard errors can have substantial impacts on wildlife management actions. As these molecular genetic techniques become more common for estimating demographic parameters, it becomes even more important for both ecologists and geneticists to recognize the unique attributes and subtleties of genetic CMR which may not be readily apparent to those unfamiliar with genetic based CMR. For instance, consider the following issues that must be deliberated when using a genetic based CMR:

- Given the potential for over-sampling (sampling an individual many times) and the cost per sample, how does a biologist prioritize which samples to analyze to minimize capture heterogeneity?
- To increase precision of estimates, is it better to reanalyze existing samples to decrease genotyping errors or to analyze additional samples already collected during previous sessions?
- How does one resolve disparate results between the laboratory and the field?
- How should ecologists select the best CMR model to use in a non-invasive design?

The overall process of using non-invasive genetic techniques to estimate population size involves three main steps: 1) designing the appropriate sampling scheme, and executing the study in the field; 2) conducting the laboratory analysis and interpreting the genetic results; and 3) using the molecular tags in a CMR framework to estimate population parameters (Fig. 1). Errors can occur at each step in the process and novel situations unfamiliar to even those knowledgeable of demographic parameter estimation can arise. If these situations are not handled and errors are not detected, they can be propagated, causing biases in the final demographic parameter estimates (Waits and Leberg 2000, Creel et al. 2003). Much attention has been paid to the limitations and pitfalls of non-invasive genetic analysis in the last 10 years (Taberlet et al. 1999, Waits et al. 2001, Mills et al. 2000, Bonin et al. 2004) and special efforts have been dedicated to improve lab protocol to minimize genotyping errors (Taberlet et al. 1996, Paetkau 2003). Recently, some attention has shifted to the CMR part of this non-invasive approach to improve the application and interpretation (Petit and Valiere 2006), and an exhaustive overview of this part of the analysis has been done by Lukacs and Burnham (2005). However, an exhaustive overview of the overall process has not been done yet, especially highlighting the critical gaps present between steps.

In this paper, we examine the three main steps of a genetic CMR approach to estimate demographic parameters, with the aim of discussing the primary limitations and sources of error. We provide a guide to effective integration of these steps to highlight issues that may be unfamiliar to either geneticists or ecologists. Our purpose is to address issues which commonly arise when wildlife biologists are initiating a molecular tagging study for the first time, focusing both on conventional issues (e.g., population closure, heterogeneity), and new issues (e.g., genotyping errors), when genetic data are used in a CMR framework. At the end of each section we offer a series of recommendations to wildlife ecologists who are relatively unpracticed with this topic and may want to use this tool to monitor populations through time. We discuss the role of ecologists throughout the process, the importance of the study design, the need for interpretation of the genetic results from an ecological prospective, the problem of error propagation, and future research needs in genetic CMR analysis.

1. Sampling design for genetic “captures” – How to improve accuracy starting with sample collection

Strong inference with genetic CMR estimates can be achieved through sampling design, reducing bias first and then increasing precision. Bias can be caused by violation of assumptions (capture heterogeneity, autocorrelation of the data, closure violation, etc.), whereas precision can be poor if the sample size (i.e. number of marked individuals and recapture rates) is small.

Reducing biases

Study designs for genetic CMR estimates have rarely been defined a priori, and often a sampling occasion is poorly identified (Lukacs and Burnham 2005). With important exceptions (e.g. bear population studies, Boulanger et al. 2006, Mulders et al. 2007), the number of sessions are typically defined a posteriori, either because samples are collected continuously with no formal sampling schemes, or because non-invasive samples are hard to accurately date, introducing uncertainty as to when the animal left the sample (e.g. Kohn et al. 1999, Wilson et al. 2003). These can cause biases in applying CMR models because model assumptions, such as population closure, can be violated (Boulanger and McLellan 2001). We strongly suggest that the first step, before initiation of non-invasive sample collection, should be to precisely define the sampling design, and the CMR model that will be used. While this advice can apply to any study, unique aspects to genetic CMR must be taken into account when designing a study. For instance, sampling designs will differ depending on whether hair or fecal samples are collected, whether samples are collected using a sampling device or simply discovered in the field (making age of the sample uncertain), whether the plan is to use a closed or open CMR model, and whether the objective is to estimate population size at one point in time or vital rates and trends over time. A robust design, which is a powerful way of obtaining both accurate single-year abundance estimates and estimates of survival, emigration rates, and trend over time (Kendall et al. 1997), is one of the most useful design in CMR studies (Lukacs and Burnham 2005) and should be considered the first choice of sampling; however, it is more complex to apply, especially in fecal sampling when samples are hard to date.

Hair samples are usually collected with hair-traps, allowing the researcher to clearly identify the number of occasions (i.e. the hair snags are checked at certain time intervals), the kind of sampling (e.g. random, systematic, adaptive), and the number of grids (i.e. power), with the ultimate goals of the sample design being robust to capture heterogeneity and maximizing geographic closure (Boulanger et al. 2004). Typically, closed CMR models have been used to analyze hair snare collections, and to estimate only animal abundance in a demographically and geographically closed population at one point in time. For instance, closed CMR model approaches are commonly deployed using data from hair snags for estimating abundance of ursids (e.g. Mowat and Strobeck 2000, Boulanger et al. 2004). The sampling design for feces collection is often harder to implement because random sampling is less logistically feasible and often more opportunistic (Matejusová et al. 2008, Marucco et al. in review). The use of fecal detection dogs may improve this in the near future for some species (Wasser et al. 2004, Smith et al. 2005, MacKay et al. 2008), because the transects used to locate feces could, themselves, be considered the sampling occasions and could be sampled multiple times. Because a strict sampling scheme is harder to conduct, open CMR models are typically implemented because they allow greater flexibility in sampling design (Prugh et al. 2005, Marucco et al. in review), and, if more surveys are conducted across time, allow estimates of population trend and other population parameters. In general, scat sampling is more suited for large scale sampling because less effort is needed to sample a broad geographic area than through hair snags collections (Flagstad et al. 2004, Mulders et al. 2007). Moreover, scat sampling is less affected by the 'trap-happy' behavioral response, typical of animals to bait posts used to collect hairs (e.g. Boulanger et al. 2006); however, the marking behavior response of animals to removed scats for collection has not been investigated yet. One potential issue with scat sampling at larger scales is meeting the assumption that every individual in the population has a non-zero probability of capture, unless the deposition patterns and home-ranges of individuals are known allowing a calibrated sampling scheme to be designed. Moreover, the actual time of deposition is usually undefined, extending the actual timeframe of sampling, and making the assumption of geographic and demographic closure questionable (Mulders et al. 2007).

Therefore, open models are usually better suited for these situations, because they do not require closure assumptions.

Most non-invasive CMR studies have identified capture heterogeneity, which biases the estimates, as one of the main issues encountered (Lukacs and Burnham 2005, Prugh et al. 2005). However, in non-invasive studies, the collection of a fecal or hair sample of the individual is considered to be the capture of the individual; therefore, it can be easier to minimize capture heterogeneity from the sampling design than in a traditional CMR study. This is especially true if deposition patterns are known and therefore the sources of heterogeneity can be addressed. With fecal sampling, problems of capture heterogeneity can be related to differing behavior of individuals with respect to the probability of finding their feces. For example, Marucco et al. (in review) found that with wolves (*Canis lupus*), strong marking behavior of dominant individuals can increase their capture probabilities, especially if their fecal signs are deposited at marking sites, which are easier to see and collect. To minimize this problem, the authors found that the best sampling design was along wolf snow tracks because they increased the probability of characterizing each individual in the pack, thus minimizing the effects of differences in individual marking behavior. Similarly, Eggert et al. (2003) followed elephant trails to collect fresh dung, but this sampling design likely over-represented larger elephants groups, whose trails are more obvious to human eye, thus causing capture heterogeneity anyway. Another source of heterogeneity that occurs when using fecal samples for recapturing individuals, is the variation in the amount of amplifiable DNA recovered from the feces from different individuals (Lukacs and Burnham 2005). If detected in the analysis, this issue could be investigated in the laboratory. Boulanger et al. (2006) found that one of the main causes of heterogeneity in recaptures (in this case with hair samples) was low capture probabilities for grizzly bear (*Ursus arctos*) females with cubs. A field solution, moving baited sites within each sampling grid, was used to reduce this capture heterogeneity-induced bias. Boulanger et al. (2004) found that calibrating the distance between traps to the grid edge could also minimize heterogeneity in recaptures. It is often hard to minimise variation in capture probabilities, but a higher effort in identifying potential sources of heterogeneity is important, because solutions to capture heterogeneity should be first considered in the sampling design in a species-specific, or study-specific manner.

Increasing precision: sample size, lab success rates, and money

In CMR analysis, increased sample size and subsequent higher capture probabilities increase the precision of estimates of population parameters, and estimate robustness to capture heterogeneity (Boulanger et al. 2004); however, in genetic CMR studies, an increase in sample size can simultaneously increase the number of genotyping errors, which, in turn, can increase individual heterogeneity in recaptures and introduce biases into the estimates (see next section). Therefore, the traditional methods of increasing sample size to increase capture probabilities and reduce heterogeneity should be carefully considered because, unless the genotyping error is zero, this solution could be ineffective and costly. Furthermore, it is unlikely that the genotyping error is ever zero (Bonin et al. 2004). This is one of the greatest dilemmas in genetic CMR sampling. The investigator should also consider that not only is sample size increased with increased sampling effort as in typical CMR sampling, but sample size can be increased by increasing lab success rates (the percentage of the samples analyzed in the lab that give positive multilocus genotyping results. This is especially true if additional funding is available for more genetic analysis (and not all collected samples are originally analyzed, which is often the case). For example, McKelvey et al. (2006) found out that collecting Canada lynx (*Lynx Canadensis*) scat and hair samples during winter snow-tracking sampling was more efficient than during summer sampling with hair-snaring techniques. This was likely due to fresher and well preserved winter DNA samples that increased lab success rates, and to snowtracking sampling which served as an effective screening mechanism that minimized DNA analysis from non-target species optimizing the use of funds for laboratory analysis.

Investigators should plan an annual budget based on a desired level of precision and number of recaptures, taking into account the average success rate of the given lab for a given species in a given area, and considering that over-sampling can have an important consequence for modelling genetic CMR data because each sample has a risk of creating a new individual if genotyping error occurs, which can inflate the population size estimate (Creel et al. 2003, McKelvey and Schwartz 2004). If laboratory success rates are not known, we recommend a pilot study, a critical step that is often overlooked in non-invasive CMR applications (Valiere et al. 2007). After genetic samples are collected, investigators should send to the genetic lab those feces or hairs with a higher probability of amplification (e.g. fresh feces collected in the winter on snow give a higher lab performance - Lucchini et al. 2002). We suggest collecting a large number of high quality samples during the sampling occasions, more than you expect to initially analyze in the lab. One of the important differences of this approach is that it is always possible to increase the number of recaptures later, if the samples have already been collected, by analyzing additional samples in the lab from the sampling occasion of interest. The desired number of recaptures and the level of precision should be defined in advance, depending on the goals of the study. If the species of interests is characterized by high capture probabilities, it is possible to reduce effort designs and number of sessions, which will decrease overall costs, but at the same time will reduce precision and estimator performance (Mulders et al. 2007). In some cases, it could be more important to invest new funding in additional lab analyses to increase recapture rates and decrease standard errors, rather than perform new analyses on a new sampling occasion. However, if there are no biological reasons to suspect heterogeneity problems in the sampling design, the presence of recapture heterogeneity in the data can be strong evidence for the presence of genotyping errors (McKelvey and Schwartz 2004). If this is the case, more effort should be invested in additional replicates of current samples rather than analyzing new samples.

Synthesis of recommendations:

i. Clearly define the study design and objectives prior to collecting samples. This includes taking into account the biology of the species, the sampling scheme given the field logistics, the CMR models to be used, and the number of samples expected to be analyzed in the genetic lab to reach the desired level of recaptures while avoiding over-sampling.

ii. Investigate the possible violations of CMR model assumptions inherent in the non-invasive method of choice, and control them from the sampling design first, if possible.

iii. In particular, minimize problems of possible capture heterogeneity directly in the field sampling design, investigating possible sources of this bias (e.g. in fecal deposition patterns). However, if this problem is hard to avoid, choosing a statistical model robust to variation in capture probabilities is crucial to minimize bias.

iv. Plan your funding for a desired level of precision, considering the field effort to collect samples, the lab success rate, and the issue of minimizing genotyping errors.

2. Laboratory analysis and interpretation of genetic results

The major biases and violation of assumptions in genetic CMR estimates stems from genotyping errors, a phenomenon that can occur when working with low quality DNA samples, such as feces and hair. Two main types of genotyping errors occur: allelic dropout which occurs when only one allele of a heterozygous individual is detected, and false alleles where an artifactual band makes a homozygote appear to be a heterozygote (Pompanon et al. 2005). In addition to these errors, which are inherent in the system, there are human errors such as scoring and transcription errors, which occur when technicians mis-interpret gel images, and mis-record those data into databases. Partial null alleles, which occur when there is a mutation at the priming site sequence causing primers to sometimes not attach to certain individuals, are another less common source of genotyping error (O'Connell and Wright 1997), but have been often overlooked (Wagner et al. 2007). Most commonly, genotyping errors create the appearance of additional individuals in the population that will never be recaptured unless the error is exactly repeated (Waits and Leberg 2000). Therefore, genotyping errors cause overestimation of abundance, because not only does it increase the minimum population size estimation, but it also biases low the probability of recapture, thus violating a main assumption of the CMR approach (McKelvey and Schwartz 2004, Lukacs and Burnham 2005). Partial null alleles instead create false individuals that can be recaptured, because they produce a systematic error which sometimes produces the same false individual (Marucco et al. in review).

Another problem, called the shadow effect, can occur when individuals share the same molecular tag due to using an insufficient number of variable molecular markers (Mills et al. 2000). When the shadow effect is present, individuals cannot be distinguished from each other (Mills et al. 2000), leading to underestimation in population size. Small amounts of shadow effect and genotyping error in the dataset can have large consequences for CMR estimates (Waits and Leberg 2000, McKelvey and Schwartz 2004). Fortunately, underestimation of abundance caused by the shadow effect can be identified using population level statistics such as probability of identity (PI) and probability of identity of siblings (PI_{SIB}), and eliminated by increasing the number of variable molecular markers used to produce a molecular tag (Waits et al. 2001). One problem with using the probability of identity statistics is that they are population level statistics which assume no inbreeding, or population structure, and most populations have some undetected level of subdivision, whether created by landscapes or social dynamics (Ayres and Overall 2004). Ayres and Overall (2004) developed a new and more robust probability of identity test (PI_{AVE}) which take into account these factors, as well as the relatedness structure of the population. However, these statistics are seldom used in the literature and have not been thoroughly evaluated in natural populations. Overestimation of abundance due to genotyping error is much more difficult to control and can cause vast overestimation of population abundance. For example, Creel et al. (2003) compared non-invasive fecal DNA results to a known population, and found that genotyping errors caused wolf population estimates to be biased by 5.5 times the real population size.

Lab protocols and techniques are constantly improving and this includes developing systems which minimize errors (Miller et al. 2002, Valiere et al. 2002, Paetkau 2003, Kalinowski et al. 2006). Several genetic approaches to minimize errors in the lab have been proposed: the multiple-tube approach (Taberlet et al. 1996), simulations to identify errors and to quantify them (Miller et al. 2002, Valiere et al. 2002, McKelvey and Schwartz 2004, Kalinowski et al. 2006), intensive error checking which involves scrutiny of pairs of genotypes which differ by only one or two alleles (1MM and 2MM pairs), and then subsequent discarding of poorly performing samples (Paetkau 2003), quantifying species specific DNA prior to use, and several other approaches (reviewed in Bonin et al. 2004, Pompanon et al. 2005). The more approaches used will detect more errors. While all these techniques to detect error are rapidly improving, we can still expect a small percentage of residual errors in the final dataset, and a clear and universal way of estimating the error is not yet available. Generally, the level of dropout errors or false alleles is estimated from the ongoing lab analysis (Broquet and Petit 2004) and refers to the documented error already removed in the lab. Virtually every final data set obtained by genotyping likely contains some residual errors (Bonin et al. 2004); these errors should not be ignored because they may bias the final results.

We suggest acknowledging that genotyping errors might not be completely eliminated from any lab protocol, and then working with the lab to assess the quality of the final multilocus genotypes to have the most reliable results for subsequent CMR analysis. This can be achieved by using sample quality quantification methods, such as the one suggested by Miquel et al. (2006) which provides "quality indexes" for each sample and genotype based on the sample's performance during multiple analyses of it. However, we believe the best check on laboratory errors is through the use of independent field data and the foresight to preserve some of the laboratory budget to re-examine any samples which produce results incongruous with field information. This is an important and critical step before starting the CMR analysis. For example, wildlife biologists could compare the genotyping data to Geographic Information System (GIS) data (Smith et al. 2006), radio tracking information, and the behavior and ecology of the study species to find incongruities in the data. The genotypes that cause these incongruities should be reanalyzed. Smith et al. (2006) established a series of GIS based rules that examined distances between collected scats and territory sizes on California kit fox (*Vulpes macrotis mutica*) to check their genetic results. Marucco et al. (in review) used a similar technique for wolves in the Alps.

When incongruence between the lab and the field data occur, several solutions exist. The best option may be to perform more replicates in the lab until results are deemed reliable. A second option is to remove the sample from the analysis, but the discarded samples might not be random with respect to identity, thus introducing biases into the population estimate (Creel et al. 2003, Lukacs and Burnham 2005). A third approach is to consider it a low quality or uncertain sample and use it in the analysis as such (obtaining a range of genotypes, and thus subsequent CMR analysis, with and without uncertain samples). In general, using field data with genetic data means that ecologists must have a solid understanding of genetic techniques, while geneticists must have a feel for how the data were collected and the assumptions of the methods, and fully recognize the final ecological and management implications of data uncertainty. Furthermore, ecologists must understand that genotyping errors are inherent in the system and can not be totally removed; likewise, geneticists should not be offended by being questioned about particular samples. Overall, close collaboration between ecologists and geneticists is fundamental to defining species-specific protocols for assessing quality in genetic analysis.

Synthesis of recommendations:

i. Adopt multiple approaches to detect genotyping errors in the lab.
ii. Acknowledge that genotyping errors might not be completely eliminated.
iii. Use the wildlife biologist's knowledge of the study species and field data to pinpoint genetic results incongruous with the field information.
iv. Consider additional analysis of troublesome or ambiguous samples.
v. If a few ambiguous samples are still present in the final dataset, assess the quality of the final multilocus genotypes by using sample quality quantification methods and independent field data, or remove the samples.

3. CMR analysis of a non-invasive genetic dataset

Non-invasive samples can be collected as part of a species detection study (e.g. McDaniel et al. 2000, Zielinski et al. 2006); these samples are typically not useful for abundance estimates, because of the ad hoc manner in which they are collected and often the small sample size (especially in terms of recaptures). Rarefaction curves might be used in this case (e.g. Kohn et al. 1999, Eggert et al. 2003). Miller et al. (2005) developed a method based on a simple urn model containing individuals of two capture probabilities, which can be applied to estimate population size when dealing with very small populations (< 100 individuals). However, if individuals are sampled often enough to estimate recapture probabilities, CMR analysis should be applied. CMR approaches strengths are that they can account for the sampling design, are designed to study the process that generates the data (Lukacs and Burnham 2005), and can be more efficient in the data analysis producing robust estimates of abundance with given levels of precision (Nichols 1992).

The CMR model should have already been selected in relation to the sample collection design applied (see above). Lukacs and Burnham (2005) provide an exhaustive overview of this topic, considering the pros and cons of using a closed model, open model, or a robust design. In any case, ecologists should be cognizant of the robustness of their methods, know the extent to which assumptions are violated, and the extent to which the results are likely to be affected in the face of such violations (Begon 1983). Before running analysis of population size estimates, the dataset should be checked for capture heterogeneity, and any other assumption, such as population closure. In particular, with genetic datasets, other important assumptions, such as no animals lose their marks and that all marked animals are correctly reported (i.e. no genotyping errors), should be tested. Unfortunately, few models have a goodness of fit test developed which can be assessed in a standard way, such as the Cormack-Jolly-Seber (CJS) model (Lebreton et al. 1992, Lebreton et al. 1993). Beside model choice, two main issues still remain in genetic CMR modelling: how to handle the presence of genotyping errors in the dataset, and how to handle the individual heterogeneity in recaptures, if present.

Modeling genotyping error

CMR analysis can be the last check of the genetic dataset. In fact, Paetkau (2003) and Lukacs and Burnham (2005) reported that datasets which have not been heavily scrutinized both by geneticists and ecologists are flagged either for geographical closure violation or presence of heterogeneity in recaptures (Lukacs and Burnham 2005). The apparent assumptions violations often disappear when the datasets were heavily scrutinized and errors removed (Paetkau 2003). Marucco et al. (in review) noticed that the probability of detection (i.e., the recapture rates) did not decrease with an increase in population size through time, and the estimates of population size did not increase with increases in sample size. These two indices are a good indirect estimate of absence of genotyping errors in the dataset.

We suggest two possible ways to deal with the presence of genotyping error (it can not be assumed to be zero, Bonin et al. 2004). If it is believed that the error rate can be considered negligible, then conduct the analysis without including the genotyping error rate. Subsequently, it is advisable to simulate different levels of error to evaluate the potential impact on the estimates of population size. This simulation allows one to determine if indeed the error rate is negligible on the final population estimate. A second option is to use CMR models that have been developed to include a parameter that estimates the genotyping error rate (Lukacs 2005). So far these models have been developed only for closed population models and robust designs, and rely on multiple assumptions, such as that the shadow effect does not exist, that two genotyping errors are never the same, and that a genotyping error does not produce the same genotype as an existing "real" individual (Lukacs and Burnham 2005). One further potential problem with these models is that they cannot separate the effects of closure violation from potential genotyping errors: both can lead to an overabundance of individuals captured only once. Unfortunately, closure is often violated to some extent; hence, these models are only useful if a lab-based residual error rate exists (which is often difficult to estimate). Moreover, the models have a problem with the model structure and identifiably of the parameters (Yoshizaki 2007). Other corrections have been developed for errors in identification using not-DNA natural markings (Stevick et al. 2001), or for tag-misreading (Schwarz and Stobo 1999), but these methods account only for false negative errors in identifications, which only decrease the probability of detection, and do not apply to genotyping errors, which also produce false individuals caught only once, or false individuals caught multiple times. Genotyping errors lead to different patterns of misidentification in CMR data (see section 2), and their effects on estimates differ depending on how and which errors are introduced into the data. Building an appropriate model for CMR data with genotyping errors requires a clear understanding of the misidentification mechanism; when different patterns of misidentification occur simultaneously (which is often the case), it is very difficult to build a likelihood-based model to analyze CMR data. Simulations of the misidentification patterns are now a good solution to explore the effects of the simultaneous presence of different types of errors. Yoshizaki (2007) has developed models for performing such simulations; however, this topic is open for further research.

Dealing with individual heterogeneity, if present

It is important to first try to resolve individual heterogeneity issues with an optimal sampling scheme and removing or minimizing all the residual genotyping errors in the lab. If individual heterogeneity is still a problem, then the solution is to model it, which is one of the most difficult problems facing estimation of animal abundance (Pledger and Efford 1998, Lukacs and Burnham 2005, Prugh et al. 2005). Studies should be designed to ensure that sample sizes (i.e. capture probabilities) are high enough so that models robust to capture heterogeneity can be used. The key to this is ensuring adequate trap encounter rates. In a simulation study, Roon et al. (2005) found out that estimators such as Mh-Jackknife or Mh-Chao are highly sensitive to the probability of recapture and thus may exacerbate the impact of genotyping errors, suggesting that heterogeneity estimators in closed population models should be used with caution in non-invasive genetic studies. A flexible framework of likelihood-based models which allows for individual heterogeneity in survival and capture rates have been developed for open CJS models (Pledger et al. 2003). Models that take into account extreme cases of heterogeneity resulting from the capture of animals just passing through a population of resident animals (i.e. transients) are another solution to this problem (Pradel et al. 1997a, b). In other cases, solutions can be built in a case-specific manner. For example, in estimating the number of North Pacific mink whales (*Balaenoptera acutorostrata*) entering trade in wild meat markets using DNA targeting and CMR analysis, the decreasing quantity of meat of one individual present in the market was modeled with an exponential decay function (Baker et al. 2007). Another source of capture heterogeneity could be caused by a variable exposure of individuals due to the location of their territories relative to the trap array locations, which can also violate the assumption of geographical closure (Boulanger et al. 2004), with the effect of biasing the estimates of abundance high over the area of trapping array. Gardner et al. (in review) proposed a solution to this problem, and developed a hierarchical spatially-explicit CMR model for estimating density from trapping arrays of arbitrary geometry, which contains explicit models for the spatial point process governing the distribution of individuals and their exposure and detection by traps.

Finally, we found that some unique situations could occur with genetic recaptures which are difficult to analyze: the recapture of an individual (e.g. from collected feces) after the individual is found dead, or the dead recovery of a genetically marked individual that has never been captured before from scats or hairs (from any dead recovery we can extract DNA from the tissue sample, thus having a molecular tag from a first captured dead individual). So far, these kinds of data have been either discarded or lumped together into a single detection to use the standard CMR techniques. Solutions have been developed only for the multiple detections within a sampling occasion, which is a common problem in non-invasive sampling. One recent approach is the ability to estimate population size from single sampling sessions using maximum likelihood or a Bayesian estimator (Gazey and Staley 1986, Petit and Valiere 2006, Puechmaille and Petit 2007). Lukacs (2005) developed alternative methods for using multiple encounters of an individual within a sampling occasion to estimate population size. These models rely on assumptions which are often hard to meet such as assuming a stable and closed population, and not allowing for any form of individual heterogeneity. Moreover, these sampling methods are limited because they can only be used to estimate population size, and do not allow estimation of survival or other demographic parameters that can be estimated with a multi-session method (Lebreton et al. 1992). In fact, as more surveys continue across time, the desire to estimate more population parameters, important for management and conservation implications, will increase.

Synthesis of recommendations:

i. Assess the fit of the CMR model and the extent to which its assumptions are violated.
ii. Acknowledge the possible presence of genotyping error and consider how various levels and types of genotyping error will impact the final demographic estimate, if at all, or try to use newly sophisticated models that incorporate genotyping errors, taking into consideration that some error patterns have not been considered yet.

iii. If individual heterogeneity is present, try to model it with respect to the sampling design.

Management implications and future needs

Genetic CMR is a highly promising tool to estimate population size and monitor populations through time (Schwartz et al. 2007), but these are just two of the possible applications. As this field of genetic CMR advances, it will allow estimation of survival rates, movement or transition rates, recruitment, and population growth (Nichols 1992). Moreover, recent multistate CMR models (Lebreton and Pradel 2002) are a promising tool for handling heterogeneity of capture and for investigating spatial aspects of metapopulation dynamics of elusive species which have not been available to scientists previously.

However, limitations and specific sources of error are present using a non-invasive dataset in a CMR framework. We emphasized the problem of looking at errors independently, because errors accumulate and can propagate. Improving the accuracy of the sampling design, developing species-specific protocols for specific objectives, improving genetic lab protocols, and the ecological interpretation of the genetic results will additively improve CMR estimates (Fig. 1). This process requires a strong collaboration between geneticists, wildlife ecologists, and statisticians knowledgeable in capture-recapture, especially if the population issues are complex. Every step of the process can produce errors without the knowledge of the other professionals, and thus the final population estimates could be biased and imprecise. This can have huge implications for management of rare species, especially if standard errors of estimates are large (Begon 1983).

Future efforts should be focused on finding the best sampling design and the best approach for each study species and situation, starting with data collection to minimize the error that will be otherwise carried forward, instead of only looking for solutions at the end and attempting to model the problems. It is fundamental to quantify in a standardized way the residual genotyping errors and better use the ecological knowledge in the error checking process. Even if the first two parts of the process are conducted properly, modellers should still try to solve individual heterogeneity problems and incorporate the different patterns of residual genotyping errors in models, topics currently of interests to researchers.

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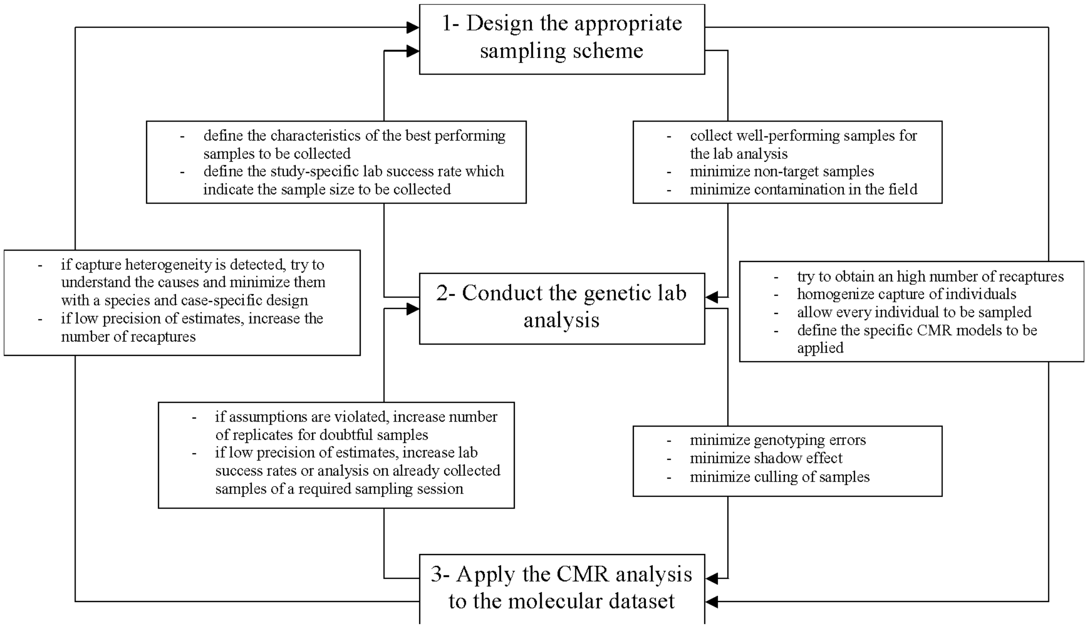

Fig.1. A diagram showing the three main steps of the non-invasive CMR approach, and the improvements that each step can make in increasing accuracy, and optimizing performances and effort, of other steps.

CHAPTER 4. OCCUPANCY DYNAMICS OF A RECOLONIZING WOLF POPULATION IN THE WESTERN ALPS

ABSTRACT

The wolf naturally recolonized part of its former habitat in the Western Alps beginning in the late 1990s. Effective management of this protected species relies on understanding distribution, on the underlying dynamics of colonization and abandonment of portions of the landscape, and on the development of a habitat suitability model that explains these patterns. To acquire this knowledge, we used a multi-season occupancy model to estimate wolf occupancy dynamics based on a large scale monitoring program. We organized multiple visits to sites to detect wolf signs (i.e. snow-tracks and scats) following a robust design over 5 years in the entire Piemonte Region, Italy. Human disturbance ($\beta = -5.553$, SE = 2.186) and rock-area cover ($\beta = -4.129$, SE = 1.392) had negative effects on occupancy, while the presence of red deer ($\beta = 0.694$, SE = 0.306) and forested-area cover ($\beta = 0.596$, SE = 0.458) had positive effects. We documented that the wolf recolonization process was characterized by a Markovian change in occupancy and the sites were not in an equilibrium state; these characteristics are typical of an expanding population. Multi-season occupancy modelling allowed us to directly model the temporal dynamics of the occupancy process and to control for the issue of "pseudo-absence", modeling directly the detection probability. We could also model the wolf range expansion in which the processes of local extinction and colonization were influenced by the distance to existing packs. These monitoring surveys, designed to estimate detection probabilities, improved the accuracy of the occupancy estimates. These surveys also allowed inference about occupancy dynamics and the process that produce these dynamics, explicitly incorporating colonization and local extinction probabilities. This monitoring approach could be applied long term and at a large scale to provide the information needed for managing rare, wide-ranging, and elusive species.

INTRODUCTION

Reliable estimates of population distribution and assessment of occupancy trends are often necessary for effective management and conservation actions (Thompson et al. 1998). A small and restricted distribution, a distribution decline, or a highly fragmented distribution are important indices of the conservation status of a species under the Habitat Directive 92/43/CEE in Europe, the Endangered Species Act in the United States, and the International Union for Conservation of Nature's Red List (IUCN 2001). Occupancy rates and the rate of change in occupancy over time define the expansion or decline of a species range (MacKenzie et al. 2006). Moreover, analysis of distribution patterns and the development of habitat models aid conservation planning because they facilitate the development of guidelines for habitat protection, and help evaluate the suitability of currently unoccupied areas (Carrol and Johnson 2008).

Wolves recently recolonized the Western Alps through dispersal from the Apennines (Fabbri et al. 2007) after being extirpated throughout most of Western Europe during the 20th century. Management decisions that support the recolonization of this wolf population are dependent on reliable estimates of wolf distribution through time, which should allow modelling of where wolves are likely to colonize next. This information can be used to reduce wolf-human conflicts, particularly in areas where husbandry methods have evolved for most of the past 100 years without predation pressure (Kaczensky 1999). Wolves, as well as other highly mobile and territorial animals, move across many unfavorable areas, but establishment success is restricted to higher quality habitats (Mladenoff et al. 1995). Regional landscape analysis and prediction of favorable wolf habitats has been conducted both in North America (Mladenoff et al. 1995, Mladenoff and Sickley 1998, Mladenoff et al. 1999) and in Europe (Corsi et al. 1999). These researchers emphasized the importance of long-term monitoring data and large-scale analysis to solve complex spatial questions in resource conservation. In Europe, where intense anthropogenic habitat modification has occurred over thousands of years, occupancy analyses and the development of a habitat suitability model over a large scale can help managers understand and manage fragmentation issues.

Statistical techniques commonly used for developing such models may give misleading results because they fail to account for factors common in datasets for species distribution: the presence of "pseudo-absence" data, an uneven survey effort, and temporal fluctuations. Presence-absence data have been used for resource selection function (RSF) analysis (Manly et al. 2002), often applying a logistic regression approach (e.g. Mladenoff et al. 1995). This approach, however, is easy to misapply due to the influence of sampling design and interpretation of results (Keating and Cherry 2004). Approaches such as logistic regression may yield biased results when applied to species presence-absence data in which the species is not truly absent from all sites at which it goes undetected (Hirzel et al. 2002, Keating and Cherry 2004, Gu and Swihart 2004, MacKenzie et al. 2006). To address these factors, we applied newly developed occupancy models to large-scale surveys to estimate probability of detecting animals (i.e., detection probability) when sample units are surveyed repeatedly and unevenly, allowing estimation of the proportion of sites occupied considering a set of covariates (MacKenzie et al. 2002, MacKenzie et al. 2006). This type of analysis solves the problem of "pseudo-absence" by directly modelling the detection probability, and is well suited for the analysis of large-scale monitoring programs and investigation of metapopulation dynamics (MacKenzie et al. 2002). Moreover, the use of multiple-season occupancy models provide strong inference about occupancy dynamics and the processes that produce these dynamics, explicitly incorporating parameters for the vital rates (i.e. extinction and colonization) responsible for changes in occupancy (MacKenzie et al. 2003). These models directly take into account temporal fluctuations, a key component of the occupancy process, which is often ignored in habitat maps which generally adopt a static view of the relationship between a species and its environment (Burgman et al. 2005).

Sign surveys have been used to estimate spatial patterns of mammals based on the detection or non-detection of signs left by animals (tracks or feces are often used) in sampling units (Manly et al. 2002, Longoria and Weckerley 2007). This is done particularly when animals are rare, elusive, or wide ranging and therefore very hard to detect directly. Because of the potential problems with bias and inadequate power that are typical of presence-absence surveys, Strayer (1999) suggested that this approach be used and interpreted cautiously. However, occupancy models used over large spatial scales directly model and account for the probability of detecting signs, thereby improving power and accuracy of estimates (MacKenzie et al. 2002, 2006).

We applied a multi-season, large-scale occupancy analysis based on wolf sign data collected over 5 years in the Western Italian Alps to address 3 main objectives. First, we determined the primary variables which predict wolf occurrence taking into account the detection probability of wolf signs. Second, we investigated occupancy dynamics by explicitly modelling potential changes in the occupancy state of a site over time with colonization and local extinction probabilities. Third, we developed a habitat suitability map based on occupancy modelling estimates, which will be useful for management purposes.

METHODS

Study area

The study area, located in the Western Alps of the Piemonte Region in Italy, encompasses the Ligurian, Maritime, Cozie, and Graie Alps. The Piemonte Region consists of 25,388 km^2 of which 11,334 km^2 are in the Alps (Fig. 1). The Region consists of a mosaic of land management classes; however, 1,485 km^2 is protected in Natural and National Parks (Fig. 1). The alpine area is characterized by long narrow valley bottoms surrounded by rugged mountains with elevations ranging from 450 to 4664 m. Dense coniferous and broadleaf forests (e.g., *Abies alba, Larix deciduas,* and *Fagus sylvatica)* are prevalent, covering about 50% of the area; the remainder of the alpine area consists of meadows and shrubland/rock areas. The snow-season generally lasts from October-November through April-May.

Sampling design

We defined survey grid cells of 5 x 5 km over the majority of the Alps mountain range of the Piemonte Region, Italy (Fig.1). We defined the smallest possible grid cell which could be reasonably monitored with the number of observers and effort we had.

This cell size allowed us to monitor the smallest packs and individual territories and to detect territorial shifts. We used the ETRS 1989 LAEA coordinate system for the grid, which is the base system suggested by the European Environmental Agency (EEA 2006). This system is particularly useful when managing species with territories crossing European boundaries, such as the wolf in the Alps. A total of 265 grid cells were surveyed to detect wolf signs over the study area (Fig. 1). We defined a set of systematic transects within each cell to collect wolf signs (i.e. tracks or scats). Transects followed trails and roads which are generally used by wolves for their movements and intersected ungulate winter ranges and rugged areas when possible. We travelled 389 transects each month over the study area for a total of 3,115.7 km; mean transect length was 8.0 ± 4.7 km.

We defined a multi-season occupancy design (MacKenzie et al. 2006: 185) analogous to Pollock's robust design (Pollock 1982) where each cell of the study area was surveyed at least once per month, and the overall survey was repeated four times each winter. We defined 4 primary time periods each winter: December, January, February, and March (Fig. 2). The secondary periods consisted of each of 5 winter seasons, from 2003 to 2008 (Fig.2). Sites (i.e. cells) were closed to changes in occupancy within winter seasons. This is a reasonable assumption for wolves which have a slow colonization rate, especially in natural recolonization areas (e.g. Ream et al. 1991, Wabakken et al. 2001). Changes may occur between winter seasons through the process of localized extinctions and colonization (MacKenzie et al. 2006).

Wolf sign surveys

We conducted winter ski- or snowshoe-based surveys with crews of 20-30 observers (generally one observer per transect) to search for wolf tracks and scats, systematically traveling every transect of the study area each month. Observers were trained prior to each field season with an intensive field and theoretical course to increase consistency in data collection between observers. We collected every wolf scat found and recorded every wolf track encountered. A key assumption of this method is that species are never falsely detected at a site when absent (MacKenzie et al. 2002). We conducted genetic analysis on scats collected along tracks for species identification (Marucco et al. in review) to assure correct attribution of wolf signs. We could estimate the age of deposition of the collected wolf signs thanks to the presence of snow (Jedrzejewski et al. 2002), and collected only wolf signs < 5-6 days old. We considered a cell occupied if we detected at least one confirmed wolf sign (i.e. a track or a scat) in the cell in a month.

Occupancy analysis

We summarized records of wolf sign detection (1) and non-detection (0) into "detection histories" similar to mark-recapture studies ($\mathbf{h}_i$; e.g. 0011 1010 1111 0110 0010). We used a maximum likelihood modelling procedure (MacKenzie et al. 2003) that relied on detection history data to estimate occupancy (ψ), colonization (γ), extinction (ε), and detection probability (p) (MacKenzie et al. 2003). From the probability statements for each observed detection history, the model likelihood is calculated as:

$$L(\psi_1, \varepsilon, \gamma, p|\mathbf{h}_1, \ldots, \mathbf{h}_N) = \prod_{i=1}^{N} \Pr(\mathbf{h}_i) \qquad \text{(eqn1)}$$

where ψ_1 is a vector of site occupancy probabilities for the first primary sampling period, γ and ε are matrices of colonization and extinctions of "wolf presence" in sites, and p is a matrix of detection probabilities. We used a logit link function to model these probabilities as a function of measured covariates. Therefore, using the logit link, we can express the probability of site i being occupied as:

$$\text{logit}\,(\psi_i) = \beta_0 + \beta_1 x_{i1} + \beta 2 x_{i2} + \ldots + \beta_U x_{iU}\,, \qquad \text{(eqn2)}$$

which is a function of U covariates associated with site i ($x_{i1}, x_{i2}, \ldots., x_{iU}$) and the U+1 coefficients that are to be estimated (MacKenzie et al. 2006). In the same way, colonization and local extinction probabilities could be functions of season-specific covariates, while detection probabilities could be functions of season-specific covariates and also of variables that may change with each survey of a site (MacKenzie et al. 2006). This modeling procedure can be considered a form of generalized logistic regression analysis where some uncertainy as to whether an observed absence equates to a true absence occurs (MacKenzie et al. 2006).

We defined a set of *a priori,* biologically plausible models, where occupancy probabilities were modeled as functions of habitat differences among sites; detection probabilities were modeled as functions of years or months, to examine the importance of different snow conditions and effort during these time periods; and extinction and colonization probabilities were modeled as functions of distance to the closest pack and habitat (*see* covariate measurements for details). We evaluated our models using a hierarchical approach (Olson et al. 2005) where we first focused on models most likely to explain detection, colonization, and extinction probabilities. Second, we held the best explanatory variables for detection, colonization, and extinction probabilities constant while focusing on our primary goal: to determine factors influencing wolf occupancy. Models were ranked and weighted according to the Akaike information criterion (AIC, Burnham and Anderson 2002). Models with $\Delta AIC_c < 2$ were used to obtain averaged parameter estimates and standard errors (Burnham and Anderson 2002). We performed all occupancy analyses using the program PRESENCE (http://www.mbr-pwrc.usgs.gov/software/doc/presence/presence.html). Then, we tested an *a priori* process hypothesis: we expected a Markovian change in occupancy (i.e. occupancy at time t + 1 is dependent upon occupancy at time t), which is logical for a territorial and site-faithful species (MacKenzie et al. 2006) such as wolves (Fuller 1989, Ream et al. 1991). However, we also tested if the data could be generated by a random (i.e. occupancy at time t + 1 is not dependent upon occupancy at time t) or a static process (i.e. where the occupancy status of the sites does not change). We used the constrained model ($\varepsilon=1-\gamma$) to test for the random hypothesis, and the single season model to test for the static hypothesis (i.e. by constraining both ε and γ equal to zero) (Table 1) (MacKenzie et al. 2006). Then we tested if the wolf recolonization process was at a point of equilibrium. Our first hypothesis was that the wolf distribution in the Western Alps was not at an equilibrium point because the population abundance is increasing (Marucco et al. in review) and unoccupied territory is still available. To test this hypothesis, we modeled occupancy and/or colonization and extinction using year as a covariate. If, for only one of these parameters, year represents an important covariate in explaining the relative variance, then the process is not at equilibrium. To test the hypothesis of an equilibrium process, we did not use year as a covariate for any parameter (except *p*), and checked if these were better models.

Covariate measurements and GIS analysis

We measured habitat features for each cell using the same grid origin for each covariate raster map using geographic information system (GIS) software (ArcGis 9.1. ESRI, Redlands, CA, USA). We hypothesized that wolf occupancy rate (ψ) could be explained by 3 main variables: landscape, human disturbance, and presence of ungulates. To characterize the landscape of the study area following Falcucci (2007), we used the CORINE Land Cover 2000 (CLC2000, version 8/2005) at the original scale of 1:100,000 and integrated with the land cover data available from GEOSTAT produced by the Federal Swiss Statistics Office. The final dataset covered the entire study area with a cell size of 100x100 m and with 15 land-cover classes corresponding to the 2nd level CORINE nomenclature (Bossard et al. 2000). We estimated 2 covariates: forested-percent cover and rocky-percent cover, then estimated the proportion of each cover type for each 5x5 km cell. We used a dataset on roads and settlements from the Cartographic Service of the Regions, with a spatial resolution at scale 1:10,000, vectorized from a topographic CTR base, to characterize human disturbance of the study area. We then produced a human disturbance raster map of 100x100 m cell size, and we evaluated the proportion of cells containing roads and settlements for each 5x5 km cell of the study area. We defined 3 different types of disturbance: a high disturbance layer characterized by the proportion of the 100x100 m cells containing main roads and settlements; a low disturbance layer characterized by the proportion of those cells containing only dirt roads, and a total disturbance layer characterized by the sum of the preceding proportions. We also produced a ski-resort disturbance raster map for the Piemonte Region (ski) from GPS locations we directly collected in the field. We evaluated the proportion of ski-resort area for every 5x5 km cell of the study area. To characterize the presence of ungulates in the study area, we used a dataset made available by the Regional Wildlife Databank of the Osservatorio Regionale Sulla Fauna Selvatica of the Piemonte Region at a 5x5 km scale. The presence of roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), chamoix (*Rupicapra rupicapra*), wild boar (*Sus scrofa*), mouflon (*Ovis gmelini*), and ibex (*Capra ibex*) were provided. From these data, we also developed a layer of presence of cervids, considering only the areas where both roe deer and red deer were detected, and a layer of total ungulate presence, considering only the areas where the presence of all 6 ungulate species was detected. The presence of red deer in the rest of the Italian Alps was determined from Pedrotti et al. (2001) with the same approach. Elevation has often been used to predict wolf distribution (e.g. Corsi et al. 1999, Falcucci 2007), therefore we also wanted to test this hypothesis. We used a digital elevation model (DEM) derived from the USGS/NASA Shuttle Radar Topographic Mission (SRTM). This dataset has a 90x90 m cell size and a vertical error smaller than 16m. We re-sampled the DEM using the same cell size and grid origin of our study area grid, and estimated the mean elevation for every 5x5 km cell of the study area.

We hypothesized that some of the variation in wolf detection rate (*p*) could be explained by the difference in sampling effort between years and months. We placed years into three groups based on differences in effort: 2004-2005 and 2007-2009 were grouped together because they had similar and high numbers of observers; 2005-2006 had poor snow coverage and a low number of observers; the remaining years were grouped together because they had similar conditions. Sampling effort differed between months; in particular February and March, which were very similar for better sampling effort and snow coverage, were considered equal.

We hypothesized that wolf colonization rate (γ) could be explained by the distance to current wolf packs and time, which are good covariates to model range expansion (MacKenzie et al. 2006). We used the dataset on wolf packs detected over the Piemonte Region by year (Marucco et al. 2007) to obtain a layer of the Euclidean distance to the closest pack, using a 100x100 m cell size, and evaluated the mean distance to the nearest pack for every 5x5 km cell of the study area for the beginning of each field season. We therefore categorized distance to the nearest wolf pack for each field season (because wolf packs changed through the years in the area, Marucco et al. 2007). We defined 4 categories: 0 km (i.e. the cells within a pack), 0-10 km (i.e. cell close to packs), 10-100 km (i.e. short distance dispersals), > 100 km (i.e. long distance dispersals). Distance to the closest pack was used both as a site covariate and as a sampling covariate. We also used time (i.e. year) as a covariate, both as an additive effect with distance and alone.

Prediction models and validation

We investigated the predicted probabilities and evaluated their confidence intervals using the Delta method (Hosmer and Lemeshow 2000). We evaluated the relationship between the probability of habitat occupancy and the most important covariates, holding each covariate constant at its mean value.

We used the occupancy parameter estimates (ψ) of the best model to produce the habitat suitability map for wolves over the Italian Alps. We also produced 2 maps which show the levels of precision of the estimates, using ψ + SE for the maximum wolf occupancy expectations, and using ψ – SE for the minimum expectations.

We evaluated the predictive power of the habitat suitability map using an independent dataset of wolf presence (1037 wolf scats collected over the Italian Alps during winter 2008-2009). Following Boyce et al. (2002), we classified the probability of wolf occurrence into 10 equal size categories of probability map scores. The independent data distribution should be highly correlated with the habitat suitability map scores if the model is a good one, i.e. indicating that the habitat suitability model was indeed predicting the relative probability of occurrence of the wolves on the landscape. We used the Spearman-rank correlation to make this evaluation (Boyce et al. 2002).

RESULTS

Habitat Occupancy

Wolves were present and detected at 75/265 (28%) sites in 2003-04, 83/265 (31%) sites in 2004/2005, 85/265 (32%) sites in 2005/2006, 89/265 (34%) sites in 2006/2007, and 93/265 (35%) sites in 2007/2008. We did not visit all sites during every survey; an average of 85.6% of the sites were visited per month. Of the sites that were visited, 44% never had wolf sign and we detected wolf sign at 56% of the sites at least in one year. Of those sites with positive detection histories, wolves were detected in only 1 year at 34 sites (13%), in 2 years at 31 sites (12%), in 3 years at 26 sites (10%), in 4 years at 19 sites (7%), and in every year at 35 sites (13%).

Occupancy was best explained by area of rocky cover, human disturbance, and by the presence of red deer (AICw_i = 0.409); the second best model had occupancy probability explained also by area of forest cover ($AICw_i$ = 0.389) (Table 1). These 2 best models explained the majority of the variability and their $AICw_i$ was 0.798; ΔAIC of the second model was 0.10. Therefore, we model averaged these 2 best models. Human disturbance (β = -5.553, SE = 2.186) and rock-area cover (β = -4.129, SE = 1.392) had negative effects on occupancy, while the presence of red deer (β = 0.694, SE = 0.306) and forested-area cover (β = 0.596, SE = 0.458) had positive effects. The sum of the Akaike weights ($AICw_i$) for the total human disturbance covariate was 0.98, suggesting the high importance of this variable in explaining the probability of occupancy. The rocky-area cover covariate was the same ($AICw_i$ = 0.98). Forested cover had a positive effect on occupancy ($AICw_i$ = 0.41), which indicated it was less important in accurately modeling the probability of occupancy. The presence of red deer was an important variable in explaining the probability of occupancy ($AICw_i$ = 0.97); the simultaneous presence of roe deer and red deer (i.e. cervids) was not ($AICw_i$ = 0.02). We examined the negative relationship between the probability of habitat occupancy and the percentage of rock-area cover, and total human disturbance, holding each covariate constant at its mean value and considering both the absence and presence of red deer (Fig. 3). Models that included ski-resort areas, elevation, or the presence of other species of ungulates did not improve the fit of the best model.

Detection, colonization, and extinction probabilities

We had a total of 950 site detections. Detection probabilities were best explained by month and grouped year (Table 1). December and January had lower detection probability compared to February and March. Moreover, 2004-2005 and 2007-2008 were the years with higher detection rates, whereas the winter of 2005-2006 was characterized by a lower detection rate (Table 2). The $AICw_i$ of month and grouped year considered as additive effects was 100%. Clearly, there is strong support that detection probability is affected by different levels of sampling in months and years. In terms of comparing hypothesis, the hypothesis that the detection probability varied among months and years has much greater support than the hypothesis that it was constant. The model with constant detection probability, the *de facto* assumption when using simple logistic regression, has no model weight, indicating no support for this hypothesis.

Colonization probabilities were best explained by an additive effect of distance to the closest pack and year, and forest habitat coverage (Table 1). Distance was better considered in the model if it was measured as a site covariate versus a sampling covariate. Distance to closest pack of > 100 km had the lowest probability of colonization compared to the other categories, as was expected (Table 2). Year was an important variable in explaining the probability of colonization ($AICw_i = 1.00$), which suggests that the wolf recolonization process is not at equilibrium yet, as was predicted. Extinction probabilities were also best explained by the distance to the closest pack (Table 1). Distance was better considered in the model if it was measured as a sampling covariate. Distance to pack of > 100 km had the highest probability of local extinction compared to the other categories, as was expected (Table 2).

The wolf recolonization process

Consistent with our a priori expectation, changes in occupancy were best represented by a Markov process. Models with random or no changes in occupancy (i.e. the static model) had little support (ΔAIC values are greater than 62) (Table 1). Additionally, the hypothesis that these sites were not in some form of equilibrium state was well supported. The top 9 models all represented some form of non-equilibrium and had a combined model weight of 1.00; the models which represented an equilibrium state received no weight in the overall set of models (Table 1).

Wolves still have a good amount of suitable habitat to recolonize (Fig. 4), especially in the Eastern part of the Italian Alps, even if in the Central part of the Italian Alps (the large lakes area) behaves as a structural barrier which could slow down the recolonization process.

The habitat suitability model was highly consistent with the independent data on wolf presence collected during winter 2008-2009 (R=0.84, p=0.02).

DISCUSSION

Quantitative evaluation of potentially suitable habitats for animal species has become an important tool for ecological assessment and conservation planning (Burgman et al. 2001). Occupancy analysis is a new approach to model habitat suitability, which gives important insights on occupancy dynamics and the processes which drive them (MacKenzie et al. 2006). Regional landscape analysis and prediction of favorable wolf habitats have been conducted in North America (Mladenoff et al. 1995, Mladenoff and Sickley 1998, Mladenoff et al. 1999), Europe (Corsi et al. 1999), and Italy (Corsi et al. 1999, Sinibaldi et al. 2001, Falcucci 2007). These researchers emphasized the importance of long term monitoring and large scale analysis to solve complex spatial questions in resource management and conservation. Large scale and long term analyses are important to understand and manage fragmentation and habitat loss problems in Europe, where intense habitat modification took place over thousands of years by human activity. Habitat maps often adopt a static view of the relationship between a species and its environment, ignoring temporal fluctuations (Jedrzejewski et al. 2008). This static view is one of the major sources of uncertainty in habitat models (Burgman et al. 2005). Multi-season occupancy modeling allowed us to use time as a covariate for colonization, as well as to use data in a temporal detection-non detection framework over 5 years. This approach allowed us to directly model the temporal dynamics of the occupancy process and understand the mechanisms. We first documented that the wolf recolonization process was characterized by a Markovian change in occupancy, where the probability that a site is occupied in a season depends upon the state of occupancy during the last season, and not by a random or static process. This is an appropriate characteristic for a territorial and site-faithful species such as the wolf (Fuller 1989). Moreover, we investigated the system occupancy dynamics where we documented a non-equilibrium process, characterized by a slow range expansion.

Wolves move throughout the landscape, across many unfavorable areas, but establishment success is restricted to higher quality habitats (Mladenoff et al. 1995). Suitable habitat for wolves has been characterized by 3 main factors: low human impact, a high proportion of forested cover, and abundant wild prey (Mladenoff et al. 1995, Mladenoff and Sicklet 1998, Jedrzejewski et al. 2004, Potvin et al. 2005, Karlosson et al. 2007, Jedrzejewski et al. 2008). We investigated these 3 main factors in our wolf study in the Alps as possible covariates that could explain occupancy. We found that wolf occupancy was strongly negatively influenced by high human disturbance. The density of roads was a negative factor for wolves elsewhere (Mladenoff et al. 1995, Wydeven et al. 2001, Jedrzejewski et al. 2008). Dirt roads during the winter might be used by wolves for movements (Whittington et al. 2005), therefore we also wanted to determine if dirt roads were selected differently than paved roads. However, in our models dirt roads were particularly important if considered as human disturbance together with paved roads and settlements. Roads may cause a marked loss of habitat at a scale of pack territory (Jedrzejewski et al. 2008); and collisions with vehicles are a major cause of wolf mortality in the Alps (Marucco et al. 2007) and in Italy (Lovari et al. 2007). Wolf occupancy was negatively influenced by the presence of rock-cover. Wolves avoided very steep and rocky areas of the Alps, although they were not influenced by elevations found in our study. Forest-cover is selected positively, but the overall weight of the variable is low and does not appear in the best model. Also, although forested areas and elevation were considered very important in wolf models based in the Italian Apennines mountains, which are more gentle and less steep than the Alps (e.g. Corsi et al. 1999, Falcucci 2007), we found that wolves in the Alps clearly primarily avoid people and rocky areas, and then occupy the remaining forested and pasture areas, especially where red deer are present. In fact, red deer presence positively influenced wolf occupancy more than the presence of other ungulate species presence. Most eastern European research shows that among ungulates, cervids are preferred by wolves (Okarma 1995). In Apennines of Italy, wild boar is the most common item in the wolf diet (Mattioli et al. 1995, Ciucci et al. 1996); however, red deer is the ungulate species selected by wolves in the Italian Alps (Marucco et al. 2008), and the predominant food item for wolves there (Gazzola et al. 2005).

An important assumption in resource selection models is that organisms have free and ready access to all available resource units (Boyce and McDonald 1999); however, in reality two similar patches of habitat may have different likelihoods of being occupied by a species due to differences in distances or connectivity with occupied patches (Jedrzejewski et al. 2008). We included this factor by directly modeling the colonization rate. Colonization rates in our study were best explained by distance to existing packs and time, where distance to the closest pack for a site can be considered the distance to a source population. However, because territory size may be larger than the extent of our sample units, it is possible that within territory movements can result in some of the apparent extinctions or local colonizations we observed. Therefore, the distance to packs of zero or < 10 km should be interpreted as within territory movements. Overall, we found a decrease in rate of colonization as distance increased. MacKenzie et al. (2006) used distance and time as covariates for γ to indirectly account for spatial and temporal autocorrelation, and some forms of clustering may be taken into account indirectly by modeling distance, such as the packs which have territories on multiple pixels (e.g. MacKenzie et al. 2006). However, if also detection is not independent among sites, the precision of the occupancy estimates might be overstated (MacKenzie et al. 2006).

Detectability was influenced by effort. Often wolves were only detected in one or two of the repeated surveys, clearly indicating that detection probabilities were less than 1. The hypothesis that the detection probability varied among months and years had much greater support than the hypothesis that it was constant, which is the assumption when using simple logistic regression. In particular, December and January had lower detection rates than other months; winter 2005-2006 also had a lower detection rate than other years, as was predicted by the lower sampling effort during these periods. Hence, we could control for the issue of "pseudo-absence", by directly modeling the detection probability and avoiding misapplications and sampling design problems typical of a logistic regression approach applied to presence-not detected data (Keating and Cherry 2004). Our robust study design that samples factors associated with detection probabilities can serve to generate more accurate estimates of habitat occupancy and provide a better understanding of the factors that influence species distributions (Kroll et al. 2007). Wolf density can influence results of occupancy models and detection rates, and it will be a critical issue to consider explicitly in the future, likely using a multistate model with pack and lone wolves treated as different states.

The disadvantage of the multi-season occupancy method is that it required intensive data collection and a robust sampling design, which is often hard to obtain, especially at a large scale and over the long term. The unconditional model of MacKenzie et al. (2003), however, provided a great deal of flexibility in the way the data could be collected compared to usual robust capture-mark-recapture models (MacKenzie et al. 2006). In particular, the ability to accommodate missing observations and unequal sampling effort across sites allows flexibility in study design. In the future, the sampling design for monitoring wolves over a large scale should consider a lower effort than we used; the level of effort can be modeled or used as a basis for stratification. Now that we know how ψ and p vary among sites, it is possible to design a lower effort monitoring program over the Alps. Our approach can be adapted for use on other elusive and wide-ranging species in Europe and elsewhere.

Management implications

The wolf population in the Alps has been identified as a unique population segment (Linnell et al. 2007), even though it is connected demographically (Marucco et al. 2007) and genetically to the Italian wolf population in the Apennines (Fabbri et al. 2007). This is because the wolf population in the Alps is different from the Apennines in ecological, socio-economic, and management contexts (Linnell et al. 2007); it also uses habitats within different countries (i.e., Italy, France, and Switzerland). Furthermore, it is a population that is currently recolonizing a considerable amount of unoccupied but potential wolf range. Species distribution and abundance are necessary to define the conservation status of a species under Habitat Directive 92/43/CEE. The wolf monitoring approach and occupancy analysis we designed could be applied in the long term and over a large scale to guarantee the information needed to manage the species, and we stress that all monitoring should be conducted with surveys designed to estimate detection probabilities, which greatly improves the accuracy of estimates. Wolves still have a considerable amount of suitable habitat to recolonize (Fig. 4), especially in the Eastern part of the Italian Alps where more habitat is available. Therefore, measures to minimize wolf-livestock conflicts should be adopted in highly suitable areas close to packs to reduce future possible depredation events, which are usually higher in newly recolonized areas (Kaczensky 1999). The wolf monitoring approach we designed should be continued in the long term to give insights on metapopulation dynamics and possible source-sink dynamics. We documented that habitat unsuitability and fragmentation may be due to natural (e.g. rocky areas, lakes) and anthropogenic factors (e.g. urban areas, roads, human settlements and infrastructures). The persistence of wolves is highly dependent on the availability of suitable habitat for establishing territories and successful reproduction (Smith and Hellmann 2002). The landscape model we designed allows practical wolf conservation planning over the Alps.

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Motivation for running the models and hp testing	Model	ΔAIC	w_i	K
Occupancy models for best ψ and γ All Markovian and not at equilibrium	ψ(r+dtot+cervus),γ(dist-st+year+for),ε(dist-sm),p(m+gryear)	0.00	0.409	21
	ψ(for+r+dtot+cervus),γ(dist-st+year+for),ε(dist-sm),p(m+gryear)	0.10	0.389	22
	ψ(r+dtot+cervus),γ(dist-st+year),ε(dist-sm),p(m+gryear)	3.90	0.058	20
	ψ(r+dtot+cervus),γ(dist-st+year+cervus),ε(dist-sm),p(m+gryear)	5.89	0.023	21
	ψ(r+dh+cervus),γ(dist-st+year),ε(dist-sm),p(m+gryear)	5.94	0.022	20
	ψ(r+dtot+ctot),γ(dist-st+year),ε(dist-sm),p(m+gryear)	6.14	0.020	20
	ψ(for+dtot+cervus),γ(dist-st+year),ε(dist-sm),p(m+gryear)	7.41	0.011	20
	ψ(for+dtot+cervus+elev),γ(dist-st+year),ε(dist-sm),p(m+gryear)	7.79	0.008	21
	ψ(r+dtot+ch),γ(dist-st+year),ε(dist-sm),p(m+gryear)	8.08	0.007	20
	ψ(r+dtot+cervus),γ(dist-st+year+for),ε(dist-sm),p(.)	12.14	0.000	17
Random model and not at equilibrium	ψ(r+dtot+c), γ(dist-st+year),ε=1- γ,p(m+gryear)	62.00	0.000	16
All Markovian and at equilibrium Model the best γ, ε, p	ψ(r+dtot+c),γ(dist-sm),ε(dist-st),p(m+gryear)	63.77	0.000	17
	ψ(r+dtot+c),γ(dist-sm),ε(year),p(m+gryear)	90.10	0.000	17
	ψ(r+dtot+c),γ(dist-sm+year),ε(.),p(m+gryear)	90.58	0.000	17
	ψ(r+dtot+c),γ(dist-sm),ε(.),p(year)	99.81	0.000	14
	ψ(r+dtot+c),γ(dist-sm),ε(.),p(elev)	101.16	0.000	10
Seasonal occupancy	ψ(year+r+dtot+ctot),γ(dist-st+year),p(m+gryear)	124.58	0.000	20
	ψ(year),γ(dist-st+year),p(m+gryear)	171.47	0.000	17
Static model	ψ(.),p(m+gryear)	528.06	0.000	21

Table 1. Summary of Akaike's Information Criterion (AIC) model selection results for habitat occupancy by wolves in the Western Alps, Italy, 2003-2008. Model names represent each parameter (ψ, occupancy; γ, colonization; ε, extinction; and p, detection) with the relative covariates (forested-area cover (for), rocky-area cover (r), high human disturbance (dh), low human disturbance (dl), high+low human disturbance (dtot), elevation (elev), presence of reddeer (cervus), presence of cervids (ctot), presence of chamoix (ch), seasons (year), grouped years (gryear), months (m), distance to formed wolf packs as a sampling covariate (dist-sm) and as a site-covariate (st)). K, no. of parameters; ΔAIC, difference in AIC relative to the most parsimonious value; w_i, Akaike weights; -2*LogL, the -2loglikelihood.

Covariate	***B***	**SE**
Habitat Occupancy		
Intercept (ψ)	-0.327	0.474
Human disturbance	-5.553	2.186
Rocks habitat coverage	-4.129	1.392
Reddeer presence	0.694	0.306
Forest habitat coverage	0.596	0.458
Colonization		
Year1	-4.274	0.639
Year2	-3.863	0.641
Year3	-4.111	0.664
Year4	-4.114	0.644
Distance to pack - 0	3.472	0.584
Distance to pack - 0-10km	1.082	0.638
Distance to pack - 10-100km	0.044	1.069
Forest habitat coverage	1.220	0.473
Extinction		
Distance to pack - 0	-1.898	0.141
Distance to pack - 0-10km	-0.578	0.223
Distance to pack - 10-100km	-0.398	0.387
Distance to pack - >100km	0.499	0.521
Detection		
Month 1 - December	-0.326	0.144
Month 2 - January	-0.001	0.144
Month 3 - February/March	0.133	0.130
Years (Good effort)	0.336	0.143
Years (Medium effort)	0.213	0.144

* In the colonization model, the intercept corresponds to the highest distance to pack (> 100km); in the detection probability model, the intercept corresponds to Years (Low effort).

Table 2. Parameter estimates, standard errors, and 95% confidence intervals for the best habitat occupancy, colonization, extinction, and detection probability model for the wolves in the Western Alps, Italy, 2003-2008.

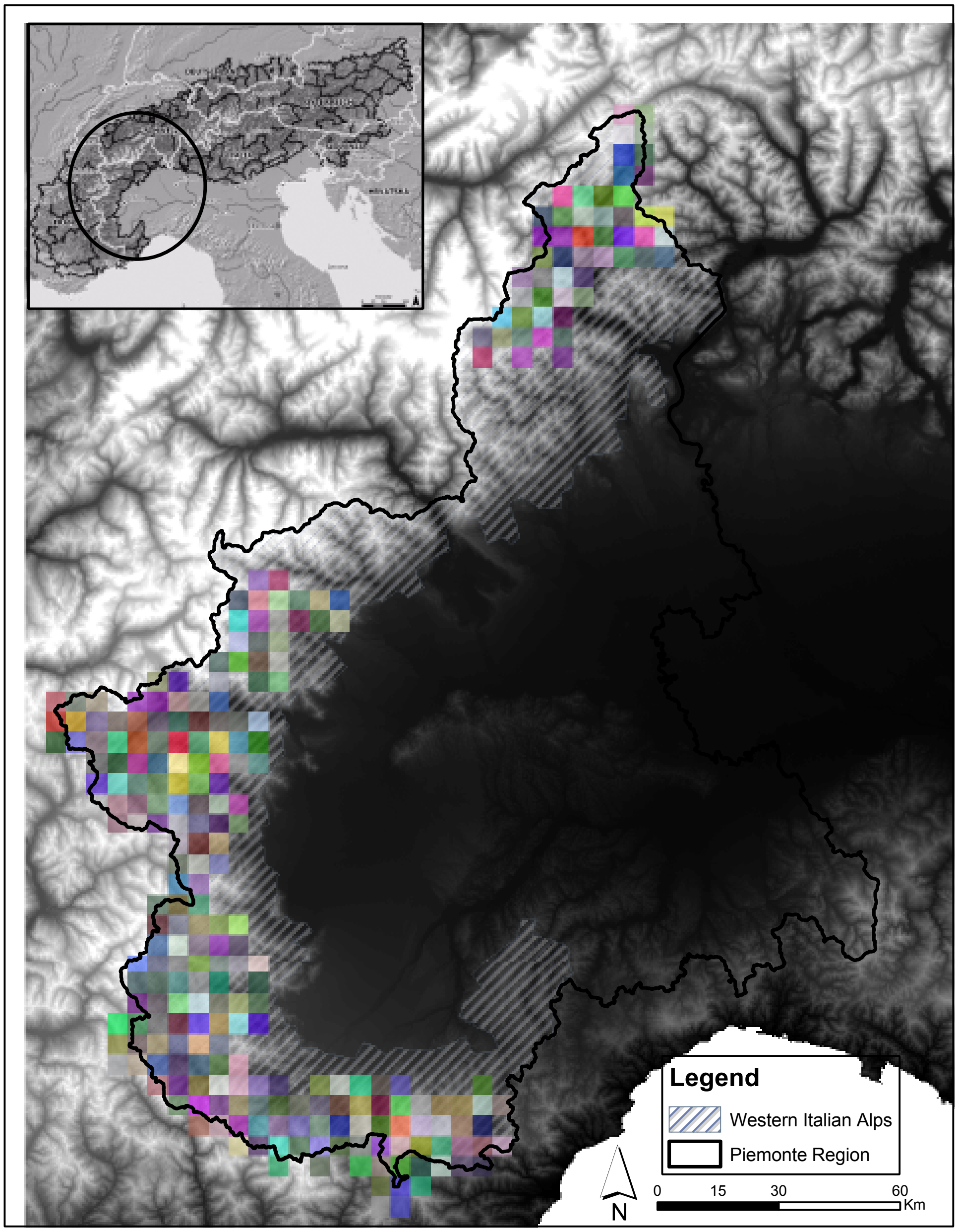


Fig. 1. Study area in the Western Alps of Italy, showing the sampling grid over the Piemonte Region.

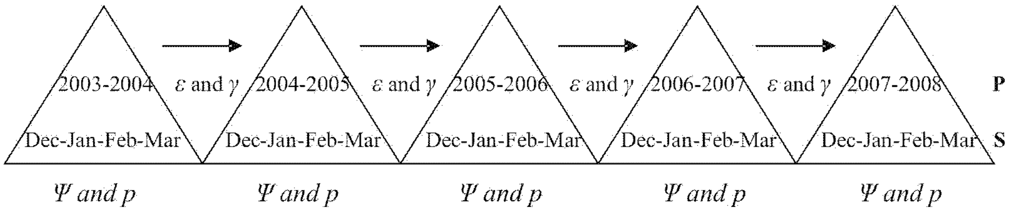
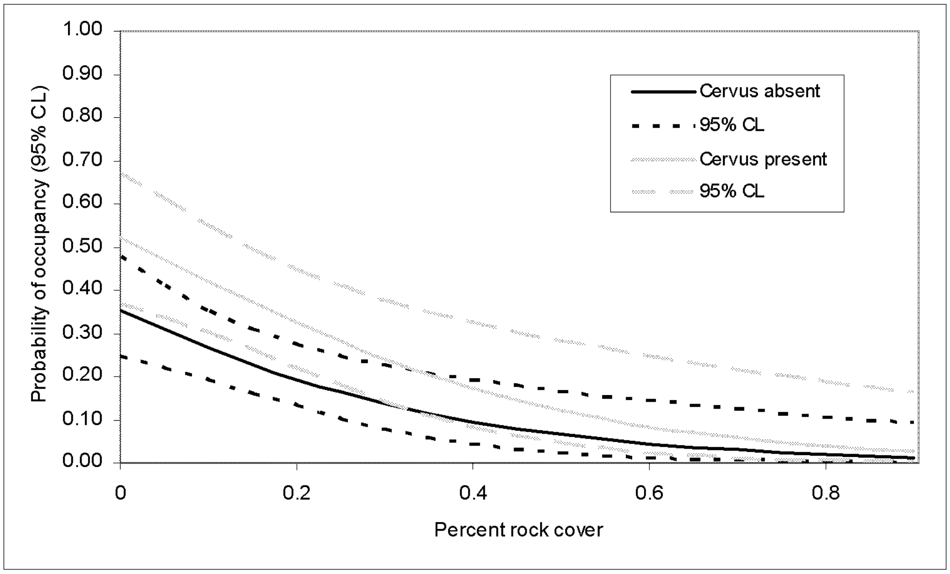
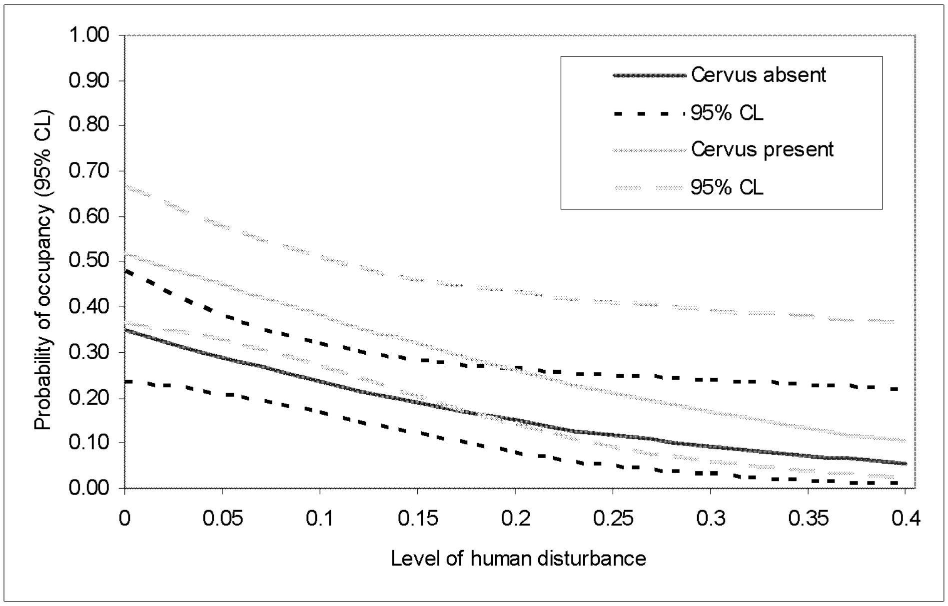


Fig. 2. Graphical representation of the multi season sampling design for wolves in the Italian Alps, where surveys represents the primary periods (P) and winter seasons the secondary periods (S) of a classical robust design. Probability of occupancy and detection are estimated within seasons, probability of extinctions and colonizations are estimated in between seasons (MacKenzie et al. 2006).


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Probability of occupancy (95% CL)
Cervus absent
95% CL
Cervus present
95% CL
0
0.2
0.4
0.6
0.8
Percent rock cover

a)


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Probability of occupancy (95% CL)
Cervus absent
95% CL
Cervus present
95% CL
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
Level of human disturbance

b)

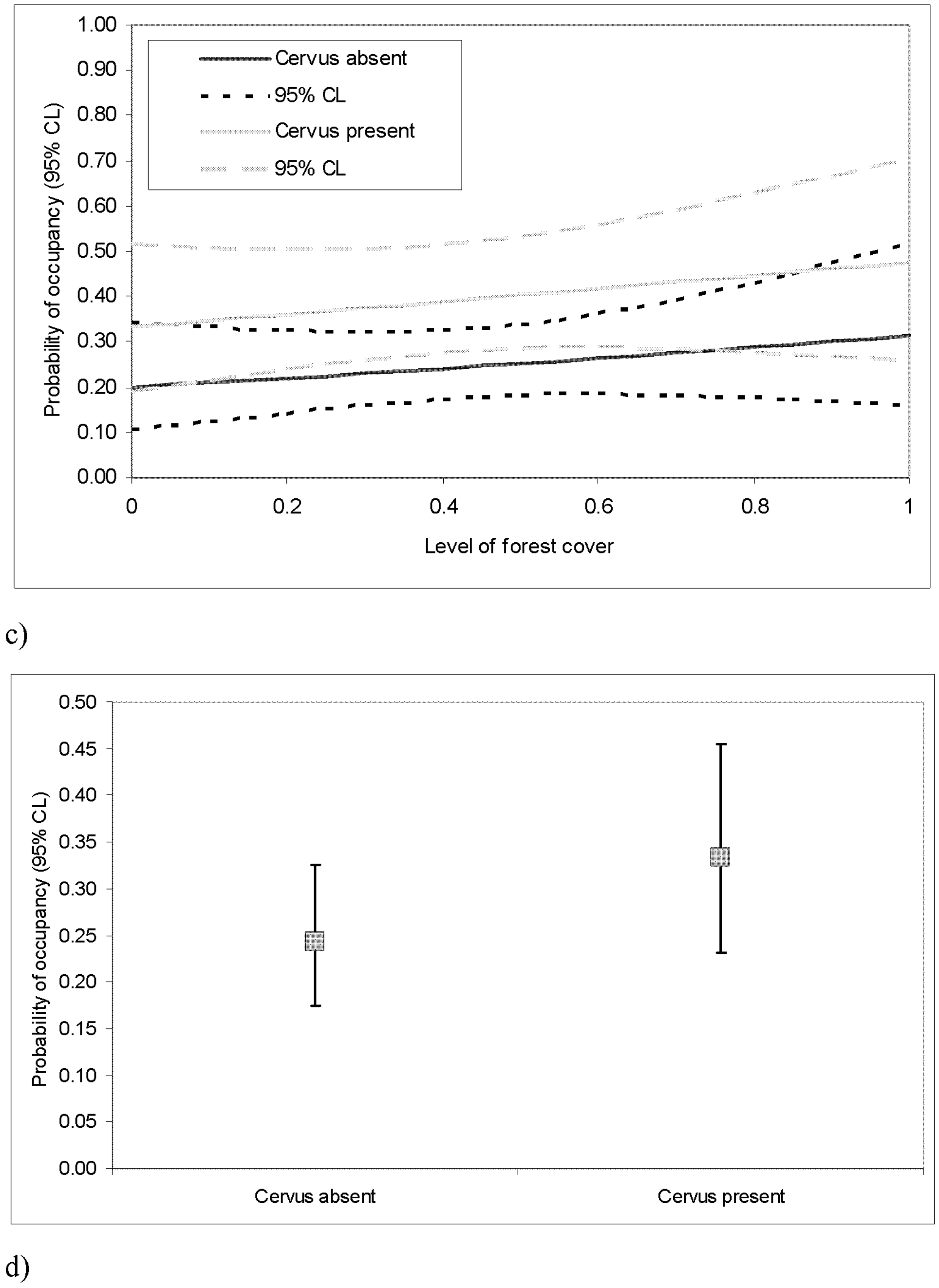


Fig. 3. Predicted probability of wolf occupancy and 95% confidence limits as a function of rock-cover percentage area (a), percentage of human disturbance (b), forest-cover percentage area (c), and presence or absence of red deer (d), after accounting for each other variable, and detection probabilities, in the Western Italian Alps.

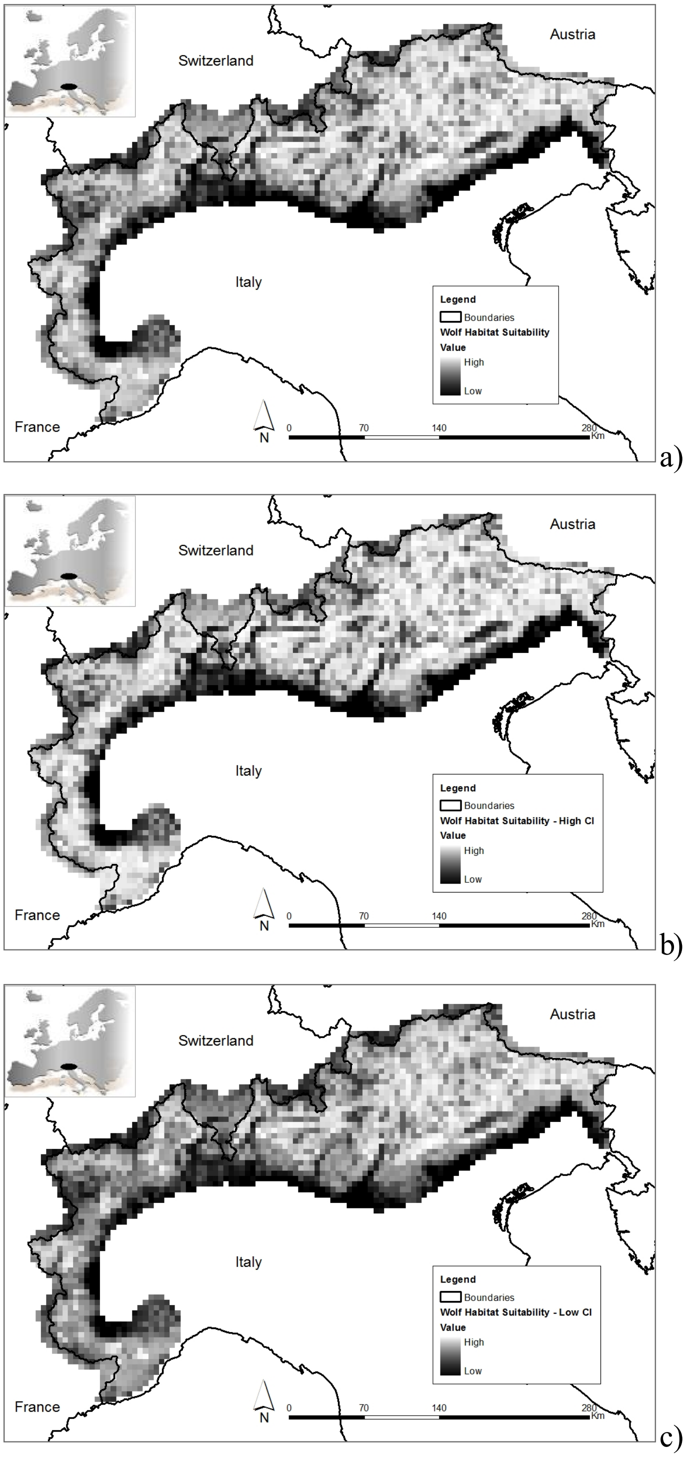


Fig. 4. Predicted wolf spatial distribution of the probability of site occupancy for wolves in the Italian Alps, as derived from the parameter estimates of the best multi-season occupancy models (a), and levels of precision of the map, using the standard error (SE) of the occupancy parameters (b and c).

CHAPTER 5. PREDICTING SPATIAL AND TEMPORAL RECOLONIZATION OF SMALL POPULATIONS: WOLVES IN THE ITALIAN ALPS

ABSTRACT

Wolves recently recolonized the Western Alps through dispersal from the Apennines. We analyzed the recolonization process in this fragmented landscape using a spatially explicit, individual-based model (SE-IBM) based entirely on information collected through a 10-year intensive study of this wolf population. We developed the model based on demographic processes, social structure, behavioral aspects, and habitat selection of wolves. We accurately modeled the recolonization process, as evidenced by the validation process, and then predicted wolf pack location and numbers, along with wolf population size, over the Italian Alps in 2013, 2018, and 2023. We predicted 25 packs (95% CI – 19, 32) in 2013, 36 (95% CI – 23, 47) in 2018, and 49 (95% CI – 29, 68) in 2023. The South-Western Alps were the main source for wolves repopulating the Alps from 1999-2008. This main source area will likely be shifted to the North-Western Alps after 2008. In the Central Alps, the large lakes area may be a spatial barrier for wolf expansion given the shape and location of the lakes. In combination with the high human presence near the lakes, the recolonization process could be quite slow in this area. We predict that in 15 years the main source for wolves repopulating the Alps will move North-Central to allow the full recolonization of the Eastern part of the Alps; this area was too far away from a source population in 2008 for recolonization. Our predictive model of future wolf expansion can be used to prioritize conservation efforts, such as preparing measures to prevent livestock depredations prior to the return of wolves in specific areas, because it predicts not only where wolves will appear in the future, but when wolf packs will likely establish. Packs have different requirements and impacts on wolf-human conflicts than wandering dispersers, and their number and location estimation are keys for wolf conservation.

INTRODUCTION

Conservation strategies for species of concern have been developed using models of varying complexities, such as population models (e.g. population viability analyses; PVAs), landscape models (e.g. resource selection functions; RSFs), and spatially explicit dynamic models (e.g. spatially explicit, individual-based model; SE-IBM) (Shenk and Franklin 2001). Often, the main factors driving small populations of large carnivores to extinction are contraction and modification of their habitat and increased illegal killing by humans (Ferreras et al. 2001). The wolf (*Canis lupus*) is a controversial species (Mech 1995). In many cases wolf populations are small and protected, but pressures on authorities to define control strategies are high even when the population is smaller than a minimum viable population (Ebenhard 2000, Nilsson 2003). Models are therefore very important to guide conservation and management decisions, minimizing the ecological and economic costs of trial and error, especially for endangered species. However, many current conservation questions are complex and may require a combination of analysis on spatial reality, animal behavior, in addition to population dynamics, for an accurate assessment of the ecological uncertainty.

Population models have often been used to define conservation strategies for large carnivores (Samson 2002), including wolves. For example, Chapron et al. (2003) assessed extinction risks under different management strategies, and Nilsson (2003) investigated the effects of inbreeding depression, hunting policy, and catastrophic events on isolated wolf populations in Scandinavia. However, PVA have been criticized because they inadequately represent the behavior characteristic of the species in question (Lima and Zollner 1996), and because they are often not spatially related to real landscapes (Macdonald and Rushton 2003, McIntire et al. 2007). Population models are enhanced by explicitly including information about behavioral strategies (e.g. social dynamics) and individual variability that affect population dynamics (DeAngelis et al. 2001, Gerber 2006, Rankin and Kokko 2007). For wolves, a highly social and territorial species structured in packs with a single breeding pair, behavioral aspects affect density, home-range configurations, reproduction, and mortality (Mech and Boitani 2003). Social structure may inflate the importance of demographic stochasticity by restricting the number of breeding units to the number of social groups (Caro and Durant 1995). In such cases, social organization may increase extinction risk by amplifying demographic stochasticity (Vucetich et al. 1997). Yet, behavioral data have been rarely incorporated into population models (Gonzalez-Suarez and Gerber 2008).

Another type of model, so called the spatially explicit (SE), geographical information system (GIS)-based, landscape models can also be very useful for species management (Andriaensen et al. 2003). These models have been widely used in Western Europe to predict potential wolf distribution in areas not yet recolonized (e.g. Corsi et al. 1999, Falcucci 2007, Jedrzejewski et al. 2008). However, social and population dynamics have never been addressed in these models, and therefore no potential feedbacks within the system have been considered (McIntire et al. 2007). Additionally, no pack requirements for territorial establishment have been accounted for, implying no differences between potential presence of wandering solitary wolves and established packs. On the other hand, non spatial IBMs or PVA models do not consider the habitat constraints in which the species of interest is living (e.g. Chapron et al. 2003, Nilsson 2003), or where the specific landscape characteristics can limit the species distribution, dispersal, and expansion, especially for highly territorial animals such as wolves. In the Italian Alps, where the landscape is highly human dominated and available suitable habitat for carnivores is heavily fragmented by both topography and humans, the habitat can play an important role in reducing the system carrying capacity for wolves.

The approach and analysis we propose here, a spatially explicit, individual-based model (SE-IBM) (Turner et al. 1995, Letcher et al. 1998, Wiegand et al. 2004), circumvents these shortcomings. Because our models are set in the real Alps landscape, they are open to empirical testing and validation, allowing sensitivity analysis to investigate the roles of various population and landscape parameters. Moreover, the SE-IBM we developed is a way to link individual traits and social system complexity, such as wolf pack structure, because they directly account for how population dynamics emerge from individual behavior (Grimm and Railsback 2005). IBMs allow the effects of demographic stochasticity and the internal complexity within a population to be taken into account in a straightforward manner (Matsinos et al. 2002), and are therefore particularly well suited for small populations (DeAngelis et al. 2001).

Wolves recently recolonized the Western Alps through dispersal from the Apennine mountains (Fabbri et al. 2007) after being extirpated throughout most of Western Europe during the 20th century. To analyze this complex natural recolonization process in a fragmented landscape, we built a SE-IBM of wolf recolonization that incorporated age-based reproduction and mortality, social structure, territoriality, dispersal, and habitat selection acting on dispersal and territory location directly within the Alps landscape. The SE-IBM for the wolf recolonization of the Italian Alps is an important approach to examining the movement and demographic parameters of wolves in their real Alps landscape, allowing for system properties to emerge from the model. We provided predictions and analysis of the potential future wolf expansions to guide wolf conservation and management decisions. In particular, we:

- Created a probability map of future wolf packs in the Alps in the next 5, 10, and 15 years, together with pack numbers and other population parameters.
- Identified critical areas for wolf pack establishment which will be fundamental for future sources in the wolf recolonization process of the entire Italian Alps system.
- Analyzed whether some packs were and will be more important than others for the success of wolf recolonization in the Alps over time.

METHODS

The study area and the habitat suitability model for wolves in the Alps

The study area covers the Western, Central, and Eastern Alps in Italy (Fig. 1). This area is weakly connected in the south-west to the Apennines Mountains, the only source for the wolf population in the Alps (Fabbri et al. 2007). The connection with the Apennine population are the Ligurian Apennines Mountains, and gene flow between the Apennines and the Alps is moderate (corresponding to 1.25-2.50 wolves per generation, Fabbri et al. 2007). Because of this, we considered immigration negligible for initial purposes of our demographic submodel. In the West-Central part of the Alps, the mountains reach 4000 m. Sheep farming is widespread in the Italian Alps and livestock depredations by wolves have caused increasing conflicts over the years (Tropini 2005);

today, however, the wolf population is fully protected in Italy and legal removal is not allowed.

Marucco (2009) developed a wolf habitat suitability model for the Italian Alps (Fig. 1), applying an unconditional, multi-season occupancy model to estimate wolf occupancy dynamics and detection probabilities (MacKenzie et al. 2006) based on data collected using a robust design over 5 years in the Western Italian Alps. In the best model, human disturbance ($\beta = -5.553$, SE = 2.186) and rock-area cover ($\beta = -4.129$, SE = 1.392) had negative effects on occupancy, while the presence of red deer (*Cervus elaphus*) ($\beta = 0.694$, SE = 0.306) and forested-area cover ($\beta = 0.596$, SE = 0.458) had a positive effects (Marucco 2009). The suitable habitat model produced had a grid structure of 25 km^2, and the habitat suitability values range from 0 to 1000 (Fig. 1). The grid size we used is finer than the typical scale of a wolf territory in the Alps, which is on average 134.8 ± 53.2 km^2 (Marucco et al. 2008). Therefore, every wolf pack territory potentially consists of 4-8 cells of the habitat suitability model. We covered the entire Italian Alps range (~ 70,000 km^2) with a grid comprising 2814 raster cells. This scale allowed us to concentrate on large scale dispersal, territory establishment, and to neglect details of small scale movements within home ranges.

The spatially explicit, individual-based model

Model structure

We constructed the SE-IBM using the Spatially Explicit Landscape Event Simulator (SELES) (Fall and Fall 2001), a raster based spatial modeling framework. For model structure purposes, we used a finely divided version of the habitat suitability model (1.56 km^2 raster cells). The SE-IBM followed the fate of individual wolves and simulated mortality, social structure of packs, reproduction for the breeding pair within a pack, movement of dispersers, and home range establishment of successful dispersers in annual time steps, with a demographic submodel that simulated these life-history events. All of this occurred within the wolf habitat suitability map of the Italian Alps (Fig. 2). Our model has three main submodels: the main model for the wolf population process which follows annual survival, reproduction, and social structure formation of individual wolves (aspatial); a dispersal submodel (spatial); and a potential territory establishment submodel (spatial). The spatial submodels are both affected by the habitat suitability map and interact with the wolf population process model. The dispersal and potential territory models occur on a finer timescale, with up to 100 moves and potential territory assessments per year. The structural uncertainty (*sensu* Burgman and Possingham 2000) of the model is relatively low because the biology of wolves is well known (Mech and Boitani 2003). However, the mechanisms behind juvenile dispersal and the decision to accept a potential location as a territory are relatively unknown. This structurally uncertain component was the subject of the calibration described below.

Parameters used in the model

We used two types of parameters defined by how they were estimated: those estimated from an ongoing 10 year intensive study of the wolf recolonization process in the Western Italian Alps (Marucco et al. 2008; Marucco et al. *in review*), which began recording and monitoring since the first packs formed in the Alps (Table 1, 2 and 4), and those estimated during the calibration phase of the present model (Table 3). Data-based parameters were used directly in the model for model functioning (Table 1), used for model calibration (Table 2), or used for model validation (Table 4).

Reproduction, survival, and pack structure

Any pack that has both a male and a female will breed in a given year, however, only the breeding pair in a pack reproduces, as it was documented so far in the Alps (Marucco et al. 2008). Litter size is determined by a random draw from N_{pup} (Table 1), and the sex of each pup is determined randomly with an equal sex ratio. After birth, pups stay in the pack for at least one year; after this, young are forced to leave the pack if the pack is larger than a random draw from the maximum pack size, P_{max} (Table 1). Mortality happens every year to young, adults, and dispersers at rates m_y, m_a, m_{dpm} (Table 1, 2 and 3). Wolves older than 14 years die (A_{max}). The annual apparent adult mortality rate ($m_{app,a}$) and annual apparent juvenile mortality rate ($m_{app,y}$) were estimated from non-invasive capture-mark-recapture analysis by Marucco et al. (in review). Apparent mortality rates include true mortality and all losses from the study area (i.e., dispersals). Because dispersal mostly happens among younger wolves (Boyd and Pletscher 1999), we assumed that apparent adult mortality was equal to true adult mortality. However, we could not make this assumption for juveniles. Thus, true juvenile mortality rate was estimated during the calibration phase (described below).

Dispersal

In the dispersal component of our model, young aged 1 and 2 years old disperse and search for their own territory if forced to leave the pack, a density-dependent process defined by P_{max}. Our dispersal data comprise 23 individual wolves with known dispersal distances (Table 1). To convert these empirical dispersal distances to a mechanistic movement process for use in the simulation, we assumed that wolf dispersal occurs by a correlated random walk (i.e., direction at time t is a function of direction at time t-1) (Turchin 1998) with 2 parameters: the move step length (α; the number of cells a wolf moves per dispersal time step), and angular deviation (σ; the standard deviation of a wrapped normal distribution). Thus, the angle is a stochastic draw from a distribution, but the potential destination cell must have a habitat quality that exceeds Q_{mean}. Furthermore, because we know that there is dispersal mortality (Marucco et al. 2008), but we do not have data on its mechanisms, we assumed that the probability of mortality increases with distance and/or time traveled. Thus, we introduced a parameter for the "per move dispersal mortality rate" (m_{dpm}) to estimate within the calibration phase. Finally, in early simulation attempts, we realized that it is impossible for the simulation model to reproduce the long distance dispersal found in the data to occur if only habitat is taken into account because there is too much unoccupied habitat that is close to all source packs that there would never be long distance dispersal. We introduced a behavioral transition parameter (ζ; Table 3) to describe an initial time period (in number of steps) immediately after leaving the natal pack where the wolf will not establish a new territory, but will join a previously existing territory with a single wolf of the opposite sex, if encountered (Joining).

Territory establishment

For establishment of a new territory, every cell that is visited after the behavioral transition parameter (Table 3) (Fig.2) will be assessed for habitat quality following habitat suitability parameters ($Q_{.}$) that we derived empirically by calculating the sum of habitat suitability for all 36 known and mapped packs during 1999-2008. If a visited cell has a habitat quality that exceeds $\mathrm{Q}_{\mathrm{mean}}$, that cell will be the center of a potential territory. The individual will assess all neighboring unoccupied cells (up to an area of 404 T_{max} km^2), summing the habitat suitability values (each on an arbitrary, relative scale of 0 to 1000), until a total habitat suitability, Q_{tot}, is reached. If Q_{tot} is exceeded within the T_{max} area and no other wolf territories are present in the cells, territory establishment is successful. Once a territory is formed, the territory location is permanent unless mortality occurs to all the members of the pack.

Calibration phase

To estimate the 5 unknown parameters in the model (4 for dispersal and 1 for juvenile mortality; Table 3), we repeatedly simulated 7 years (i.e., until 2006, the date at which we had our parameter estimates) with numerous, biologically plausible combinations of these parameters and picked the best combination that minimized a cost function, thus eliminating parameter combinations that were not able to reproduce the known values (Wiegand et al. 2004). Our cost function used 2 population parameters, apparent juvenile survival rate and number of packs, and 5 quantiles of dispersal distances (Table 2). We selected these because they represented both spatial and aspatial phenomena, and included a key life history parameter, and number of packs, which is known with fairly high confidence. All simulations initiated as if it was 1999, with the known 6 pack locations, and 23 individuals of known age category (pup or adult) and social category (non-breeder or breeder) (Fig. 1). We minimized the following cost function:

$$C = \sqrt{\left(\frac{\mathrm{m}_{\mathrm{app,\,j}} - m_{app.sim,j}}{m_{app,j}}\right)^2} + \sqrt{\left(\frac{N_{packs} - N_{packs.sim}}{N_{packs}}\right)^2} + \frac{\left(\sum_{q=1}^{5}\sqrt{\left(\frac{d_q - d_{q.sim}}{d_q}\right)^2}\right)}{5},$$

where *sim* indicates the median value from 100 replicate simulations at the given combination of parameters (Table 2). Essentially, this cost function minimizes the sum of the squared deviations between the simulated and known values for apparent juvenile survival, number of packs, and the 5 quantiles of dispersal distances, rescaling each term by the known value of the parameter so proportional deviations are equally weighted.

Model testing: validation

We tested our model using parameters that were not used for model calibration (Table 4). Furthermore, we simulated until 2008 (9 years), 2 years past our calibration phase, to test our model over the longest period possible. We used spatial and aspatial parameters, at both the population and individual levels, to assess numerous types of prediction capacity. First, at the individual level, we used the known fate and territory establishment history of all wolves that died from the initial 23 wolves (N = 16). Second, we tested the model with the annual growth rate estimate evaluated independently with genetic capture-mark-recapture (CMR) data (Marucco et al. in review). Third, we assessed the wolf population estimate in 2008 obtained using results from genetic analysis on scat samples and snow-tracking data (Marucco et al. 2008). Fourth, we did a spatial cross validation test used commonly in the resource selection function literature (Boyce et al. 2002). Finally, we also calculated the likelihood and used AIC to compare the support for the spatial predictions of pack locations from three hypotheses: complete spatial randomness, a habitat-only hypothesis, and our SE-IBM. To do this, we used the pack presence or absence data from 2008, we assumed a binomial likelihood model, and required absolute prediction probabilities for each pixel in our map. These are available from our model. For complete spatial randomness and habitat-only models, we needed to estimate *absolute* prediction probabilities for each cell. To do this, all that is needed is known population size and relative probabilities. Thus, from the relative probability, habitat suitability surface, we calculated the expected probability of use as the ratio of number of pixels used ("population size") to number of pixels available (100000) and we assumed a binomial likelihood (pack presence/pack absence).

Secondary predictions and main questions

Having selected a single set of point estimates for the five parameters optimized during the calibration stage, we ran the model to investigate details of the spatial and population dynamics of the current recolonization. We then predicted the future probability of pack locations, pack numbers, and population size for years 2013, 2018, and 2023, to estimate wolf range expansion in the Italian Alps. We used the 2008 locations of the 13 known packs (Fig. 1) and pack structures as the starting state of the simulation model. We performed 100 simulations for number of packs and population size predictions, and 10,000 simulated predictions of pack locations. We developed absolute probability maps of wolf pack presence on the Italian Alps for the next 5, 10, and 15 years by summing all 10,000 rasters of pack presence (1 for pack present, 0 for absent).

We analyzed the prediction map of pack presence developed, and identified critical areas for wolf pack establishment which will likely be important as future sources in the wolf recolonization process of the entire Italian Alps.

We analyzed the extent to which some packs were and will be more important than others for the success of wolf recolonization in the Alps. We evaluated, from the two real starting scenarios in 1999 and in 2008, the mean number of successful dispersers per pack. We evaluated the relative importance of packs in 1999 in producing successful dispersers for the subsequent documented 10 years. We also predicted which packs in 2008 would be more important in producing successful dispersers for the subsequent predicted 5, 10, and 15 years.

RESULTS

Model calibration and validation

From the model calibration procedure, we selected the parameters that minimized the discrepancy between simulated and known values for apparent annual juvenile survival, dispersal distances, and pack number using the cost function (Table 3). We were able to find parameter combinations that fit perfectly for the aspatial parameters and an average of 21% deviation at each distance quantile between simulated values and known values for each parameter (Table 2).

The SE-IBM wolf model accurately captured wolf population dynamics in the Alps landscape in 2008, as confirmed by both aspatial and spatial validation. The predicted mean wolf population growth rate was very close to the estimated one from

CMR data (Marucco te al. in review) and the 95% confidence interval estimate fell within the 95% confidence interval of the simulation. The population size estimate also fell within the 95% CI (Table 4). The ages of death for the 14 known individuals all fell within the 95% confidence intervals of the predicted ones from the best model scenario (Fig. 3). Moreover, of these known-fate individuals, 3 successfully dispersed and established new packs. The model could capture exactly these processes for the 3 individuals, and in particular 95/100 times for one wolf, and 61/100 and 55/100 for the other 2 wolves.

The model was highly consistent with the independent data on wolf pack presence collected during winter 2008, as indicated by the validation of the absolute probability map of wolf pack distribution in 2008 for the Italian Alps ($R = 0.969$; $p << 0.0001$). All these data strongly validated the best model scenario.

The wolf recolonization process in the Italian Alps: questions and predictions

The number of wolf packs and wolf population size increased from 2008 through the next 15 years on the Italian Alps range (Figs. 4 and 5), probably due to the large amount of unoccupied but suitable habitat present. After the 15 years of simulation beginning in 2008, the South-Western part of the Alps reached a high-density level and likely a saturation point, while the Eastern part of the Alps still had a good amount of habitat for population expansion (Figs. 1 and 5).

The Ligurian-Maritime Alps (i.e. the South-Western Alps) was the main source for wolves repopulating the Alps from 1999-2008; the main source area for dispersers will likely be shifted to the Cozie Alps (i.e. the North-Western Alps) in the next 10 years as indicated from the predictive maps (Fig. 5). It is likely that in the future the main source for wolf repopulating the Alps will move in a northerly direction to allow the full recolonization of the Eastern part of the Alps, which is too far away in 2008 for effective recolonization. Therefore, the Graie Alps, west of the lakes close to Switzerland (Fig. 5), is a likely area important for the future wolf recolonization of the Eastern Alps.

We evaluated, from the two real starting conditions in 1999 and in 2008, the mean number of successful dispersers per pack for the next 10 years. From 1999 to 2008, wolf pack 2, followed by pack 4 (Fig. 1), produced on average the largest number of dispersers, while packs 1 and 3 the lowest (Fig. 6). The mean dispersal distances of dispersers from the South-Western portion of the Alps (packs 2,3,4,5, and 6) (Fig. 1) was 73.2 (± 13.7) km. Dispersers from wolf pack 1 (Fig. 1) had the longest mean dispersal distance of 96.3 km (52.1-172.3). From 2008 to 2018, wolf packs 4 and 11 (Fig. 1) produced on average the largest number of dispersers (Fig. 6), with a mean dispersal distance of 35.6 km (1.3-93.2), and 41.7 km (4.8-113.1), respectively. Wolf pack 1 produced more dispersers between 2008 to 2023 than between 1999 to 2008, whereas wolf pack 2 produced less (Figs. 1 and 6).

DISCUSSION

Wolves were extirpated throughout most of Western Europe during the 20th century, and just recently they started recolonizing the Western Alps through dispersal from the Apennines after an absence of about 100 years (Fabbri et al. 2007). Italian wolves are listed as endangered under the Habitat Directive 92/43/CEE, the Bern Convention (1979), as well as the International Union for Conservation of Nature Red list (IUCN 2001). The ability to project the spatial and temporal expansion of small populations is important to management and conservation (Carroll et al. 2003) and has been done for carnivores other than wolves in Europe (Kramer-Schadt et al. 2004, Wiegand et al. 2004),. We developed and validated a model which integrated wolf behavioral information into a spatial demographic model to predict the distribution and number of wolf packs in the Italian Alps. Our approach incorporated the relationships between wolf social behavior, population dynamics, and the landscape structure of the Italian Alps. This predictive model of future wolf expansion is useful to prioritize conservation efforts, such us measures to prevent livestock depredations prior to the return of wolves in specific areas or identification of important corridors and areas for successful wolf recolonization, because it related time to space producing year-specific absolute probability maps of pack distribution over the Alps.

Regional landscape analysis and prediction of favorable wolf habitats have been conducted in North America (Mladenoff et al. 1995, Mladenoff et al. 1999), in Europe (Corsi et al. 2000), and in Italy (Sinibaldi et al. 2001, Falcucci 2007). Time and population dynamics were not addressed in these models; therefore, no potential feedbacks within the system were considered, and no pack requirements for territorial establishment were accounted for, implying no differences between potential presence of wandering solitary wolves and established packs. Wolf dispersers, as well as other highly mobile animals, apparently move across many unfavorable areas, but pack establishment success is restricted to high quality habitats (Mladenoff et al. 1995). Therefore, in any wolf landscape analysis, it is fundamental to distinguish between predictions of wolf pack establishment and of general wolf presence, which includes data on wandering dispersers. Wolf populations are often quantified in numbers (Mech and Boitani 2003), which is less biologically meaningful and more prone to error than pack number estimates (e.g. 7 wolves in 2 packs have a different meaning for a population than 7 wolves in 1 pack). Moreover, in the Italian Alps, where the landscape is highly human dominated and available suitable habitat for carnivores is heavily fragmented, habitat constraints on wolf pack establishment can play important roles in reducing the carrying capacity for wolves. Therefore, we considered directly the Alps landscape in our analyses, which constrained the wolf expansion. Packs have both different spatial requirements and a different impact on wolf-human conflicts than wandering dispersers; therefore prediction of pack numbers and location is key for population management. This analysis is the first attempt in Western Europe to predict pack numbers through time, producing a probability map of future pack presence. This allows an analysis of the important present and future source areas for further wolf expansion in the Eastern Alps.

However, projecting the spatial expansion of populations is dependent on a good understanding of how the species interacts with the landscape and presents a major challenge to conservation biology (Lima and Zollner 1996). The primary problem that affects IBMs is the uncertainty regarding dispersal behavior and habitat use, which are the critical factors determining the value of the models for conservation and management (Wennergren et al . 1995, Ruckelshaus et al. 1997, Ruckelshaus et al. 1999, South 1999). Ordinarily, time, budget constraints, or limited access to empirical data prevent precise? parameterization of the model and field testing (Roloff et al. 2001). This is particularly challenging for modeling optimal reintroduction programs, when data do not exist prior to reintroduction (e.g. Carroll et al. 2003, Bar-David et al.2005). In these cases, or generally when no good sources of data exist, data from other populations or related species are used (South et al. 2000, Cramer and Portier 2001), incorporating further uncertainty into the model. SE-IBMs generally have large data requirements, which produced much criticism on the use of such models when good data on the population is missing (Ruckelshaus et al. 1997). We collected the data used in these analyses intensively in the Alps over a 10 year period (Marucco et al. 2008, Marucco et al. in review), and the habitat suitability map was derived from these data using robust occupancy modeling approaches (Marucco 2009). For the unknown structural components of our model, we had one primary assumption: dispersal is a correlated random walk described by 5 unknown parameters. The assumption that dispersal follows a correlated random walk is likely to be sufficient because it adequately describes a wide variety of movements across taxa (Turchin 1998), and estimating 5 parameters is not too onerous for the dataset that we had.

Validation of the model is important when the models are a basis for management decisions; it can also at least partially confirm the theoretical basis of a model (De Roos and Sabelis 1995). We accomplished this with data which were not used for model parameterization or calibration, and obtained sufficiently accurate fits for the wolf recolonization of the Italian Alps. Therefore, our model can tentatively serve as a tool in an adaptive management approach for species conservation, providing an opportunity to explore the future biological, logistical, and financial consequences of alternative conservation management scenarios (Macdonald and Rushton 2003) and minimizing the ecological and economical costs of trial and error (McIntire et al. 2007).

The wolf population in the Alps

The first wolf packs in the Ligurian-Maritime Alps, on the South-Western portion of the Alps, were the most important packs in producing successful dispersers during the first 10 years of the documented recolonization process. This area could be considered the main source for wolves repopulating the Alps during this time. The mean dispersal distances of dispersers from this area were shorter than the distances dispersed by wolves from the Western part of the Alps, and this is likely because the unoccupied suitable habitat was abundant in the South-Western Alps. The simulated predictions after 2008 showed a different pattern. The main source of dispersers will likely move from the group of packs in the South-Western portion of the Alps to the group of packs in the Western Alps. This transition can be seen by looking at packs in the Western Alps during the first 10 years (not very productive), and contrasting their contribution to dispersers after 2008. This prediction is likely because wolf pack density increased in the South-Western Alps and fewer free territories were available for short distance dispersals which were usually the most successful. In fact, in the same years, the average dispersal distance of dispersers for every pack increased, likely because free territories were available only at greater distances. Newly formed packs in 2008 were less successful than any other pack because the starting structure of the pack was characterized by only one pair of wolves. Therefore, these packs needed more years to be fully productive, as is suggested by the predictions. In the Central Alps, the large lakes area may be spatial barriers for wolf expansion given the shape and location of the lakes. In combination with the high human presence near the lakes, the recolonization process could be quite slow in this area. The predictive maps suggest it is likely that in 2023 the main source for wolves repopulating the Alps will move in a northerly direction to allow the full recolonization of the Eastern part of the Alps, an area which was probably too far away in 2008 for recolonization. Therefore, a likely area important for future wolf recolonization of the Eastern Alps is in the Graie Alps, West of the lakes, close to Switzerland.

Future conservation issues and management implications

The wolf population in the Alps will likely increase and occupy the Alps mountain chain, from the West to the East through time. The high dispersal capability of wolves (Boyd and Pletscher 1999) allows for long dispersal movements; therefore, solitary wolves could easily appear in the Eastern part of the Alps already in 2009. However, a group of packs must be formed in the Central part of the Alps for a successful recolonization of the entire Italian Alps, to constitute the new source for wolf repopulation of the Eastern Alps. This would allow a more likely pack formation process in the Eastern Alps, which is a much slower process than just presence of dispersers. At the same time, wolf pack density will increase in the Western Alps. Therefore, for future analysis, we should consider specific sources of mortality, which could become more important as wolf density increases locally, such as an increase in road mortality, which is likely if more packs will be formed in less suitable areas close to cities and roads, and an increase in intra-species competition, which is likely if wolf density is high (Mech et al.1998, Fuller et al. 2003, Mech and Boitani 2003). These new aspects will be important to incorporate in the future development of this model and to further assess the recolonization process because they are key components as wolf population density increases.

The wolf population in the Alps has been identified as a unique population segment, unique in its ecological and socio-economic contexts and from a management perspective (Linnell et al. 2007) because it inhabits the alpine area within different countries (i.e., Italy, France, and Switzerland). The spatially explicit, individual-based model we developed will provide important insights on how to manage this endangered species of conservation concern between countries which have different management priorities. In particular in Western Europe, where intense anthropogenic habitat modification has occurred over thousands of years and is still increasing today, a further development of this model will be important to analyze and manage fragmentation issues. Our model can serve as a tool for assisting in wolf conservation and in the future can help predict the influence that land use and future human development may have on the spatial expansion and connectivity of the wolf population in the Alps.

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Table 1. Parameters estimated from data collected during an ongoing intensive study of the wolf recolonization process in the Western Italian Alps, and used for direct parameterization of the SE-IBM model.

Parameters estimated from data	**Code**	**Statistics**	**SD**	**n**
Mean pack size detected in late winter*	P_{mean}	4.405	1.251	74
Mean number of pups per litter	N_{pup}	3.387	1.210	31
Maximum wolf older age	A_{max}	14		
Minimum value of total habitat suitability per wolf territory	Q_{tot}	89288	16655	36
Minimum value of habitat suitability per cell in the wolf territory	Q_{min}	376		36
Mean value of habitat suitability per cell in the wolf territory	Q_{mean}	840		36
Maximum territory size, km^2*	T_{max}	404		36
Annual apparent adult mortality rate**	m_a	0.18	± 0.04	87
Starting number of packs in 1999*	P_{start}	6		6
Starting location of packs in 1999*	*Snl*	Fig. 1		6
Starting social structure of packs in 1999 (each individual)*	*sss*			23

* Estimates derived from Marucco et al. 2008.

** Estimates derived from Marucco et al. (in review).

Table 2. Parameters estimated from data collected during an ongoing intensive study of the wolf recolonization process in the Western Italian Alps, and used in the SE-IBM model for calibration of the model.

Parameters	**Code**	**Statistic**	**SE**	**n**	**Calibration (95% CI)**
Dispersal distance quantiles (0.025-0.25-0.50-0.75-0.975)	d_{disp}	11.93-24.20-54.60-176.95-365.05		23	10.08, 36.76, 75.98, 143.16, 278.32
Apparent annual juvenile mortality rate	$m_{app,j}$	0.76	± 0.06	87	0.76 (0.67-0.86)
Pack number in 2006	$N_{pack06s}$	10			10 (5-17)

* Estimates derived from Marucco et al. 2008.

** Estimates derived from Marucco et al. (in review).

Table 3. Parameters and their estimated values found during calibration phase of the spatially explicit, individual based model of the wolf recolonization process in the Italian Alps.

Parameters	**Code**	**Estimated Statistic**
Actual annual juvenile mortality rate	m_j	0.449
Angular deviation, degrees	α	21.80
Behavioural Phase Transition, moves before assessing habitat for new territory	ζ	19.80
Dispersal mortality rate, per move	m_{dpm}	0.035
Move Step Length, m per step	M_{sl}	10.93

Table 4. Parameters estimated from data collected during an ongoing 10 year intensive study (1999-2008) of the wolf recolonization process in the Western Italian Alps, and used in the SE-IBM model only for validation of the model.

Parameters	**Code**	**Statistic**	**SE**	**n**	**Validation**	**95% CI**
Pack location in 2008*	*pl*			13	(see text)	
Fate of individuals	*f-ind*			16	Fig.4	
Population size in 2008*	N_{08}	68			64	32-107
CMR growth rate**	λ	1.04	± 0.28	87	1.07	± 0.07

* Estimates derived from Marucco et al. 2008.

** Estimates derived from Marucco et al. (in review).

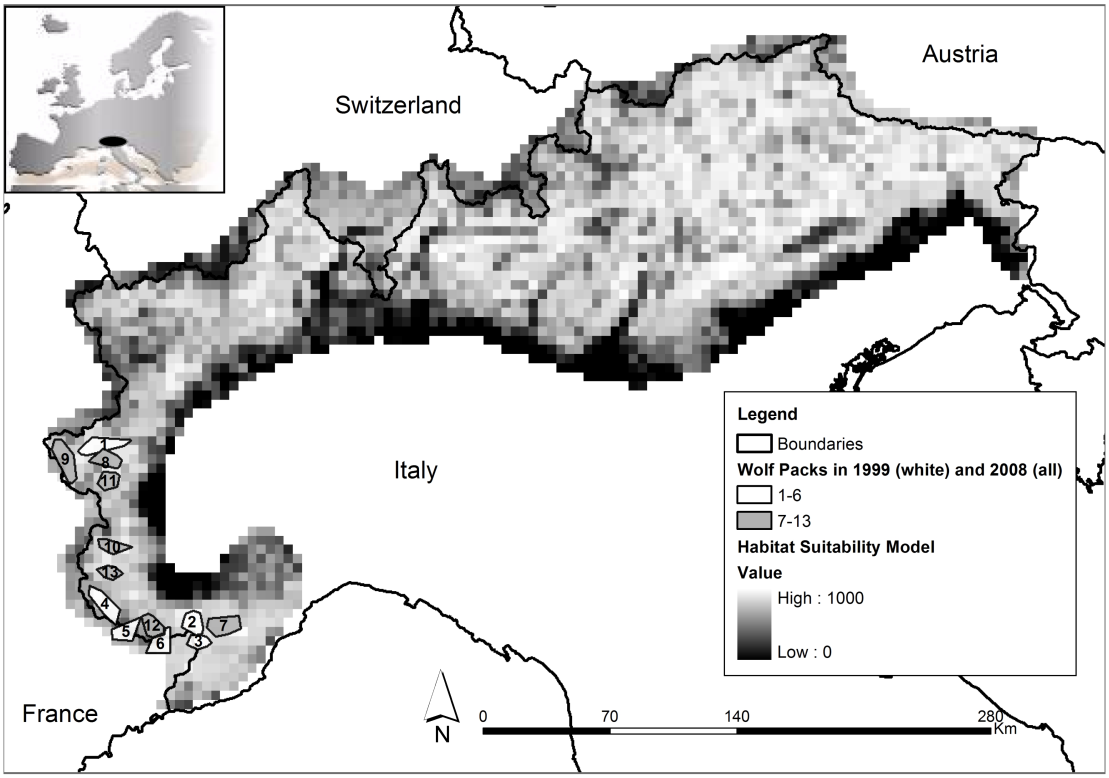


Fig. 1. Habitat suitability model for wolves in the Italian Alps, derived from Marucco (2009), and real first 6 packs locations in the South-Western Alps used as the starting state of the simulation model in 1999, and real 13 packs locations in the Western Alps used as the starting state of the simulation model in 2008.

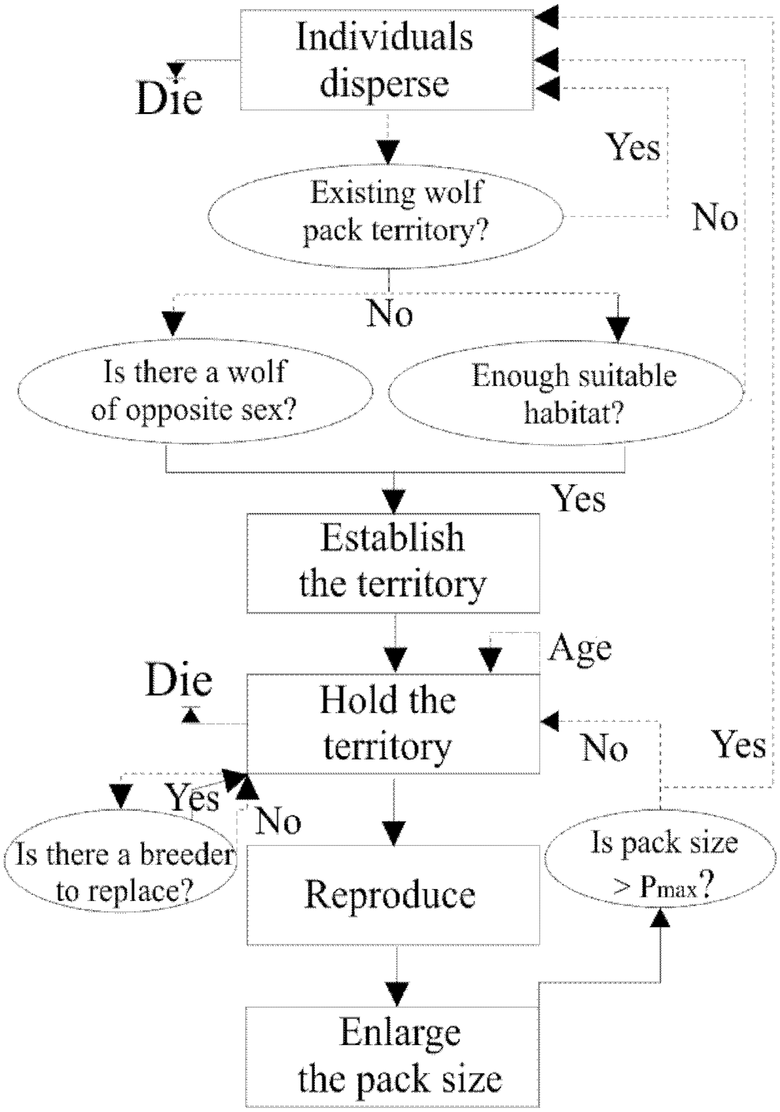


Fig. 2. Flowchart showing the way the spatially explicit, individual-based model of wolves in the Italian Alps followed the fate of individual wolves and simulated movement of dispersals, home range establishment of successful dispersal wolves, mortality, and reproduction for the breeding couple within a pack every year. Solid lines connecting boxes and ovals represent breeders, dashed lines represent non-breeders. Ovals represent questions and rectangles represent states or processes.

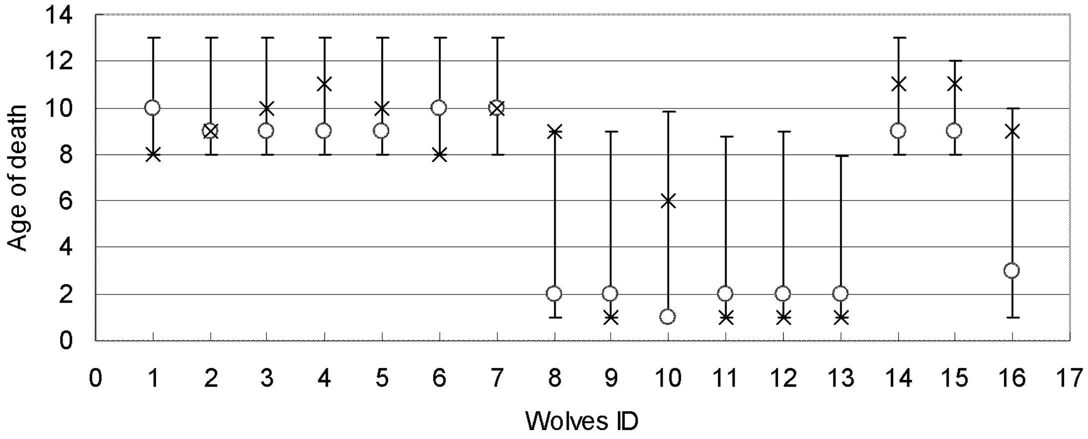
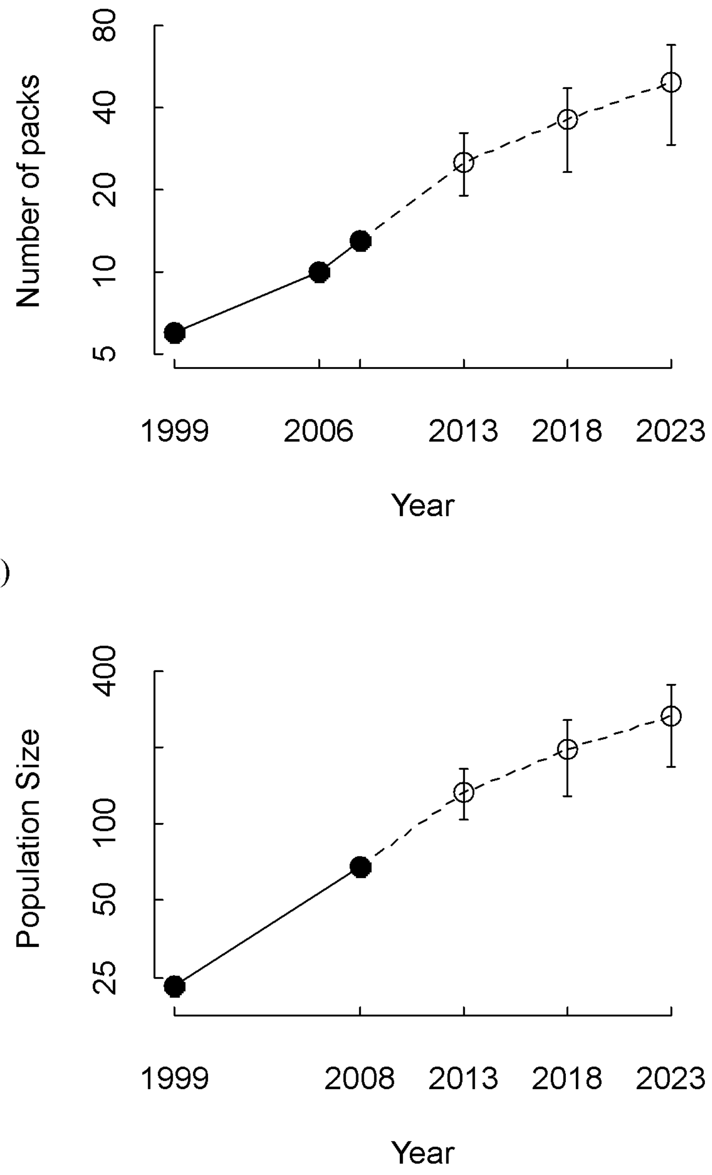


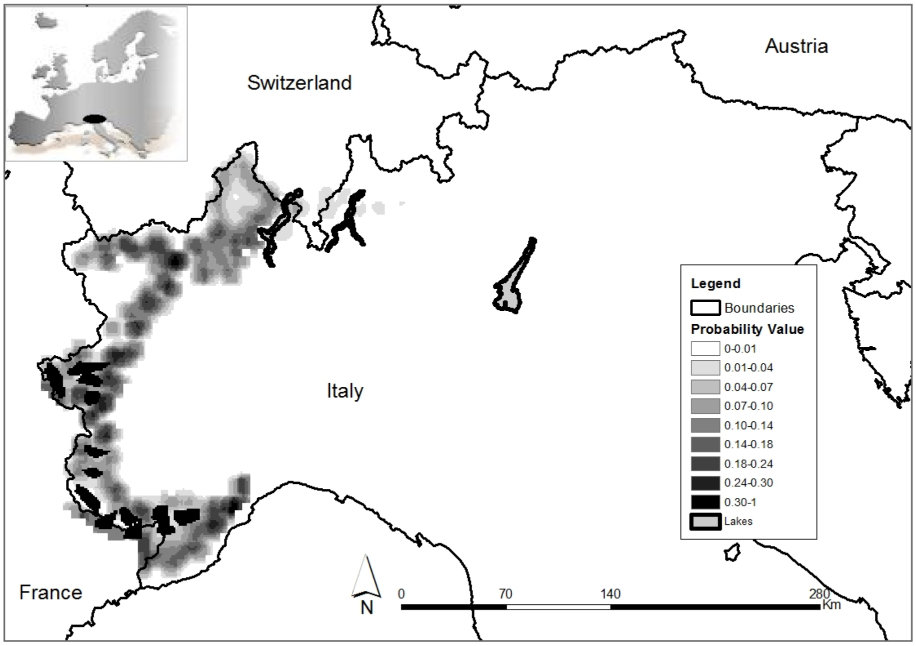
Fig. 3. Median with 95% confidence intervals of the age of death of the known 16 wolves from the starting scenario in 1999 of the 100 model simulations during the first 9 years. "X" indicates the age of death of known wolves from real data from the wolf population study in the Western Alps.



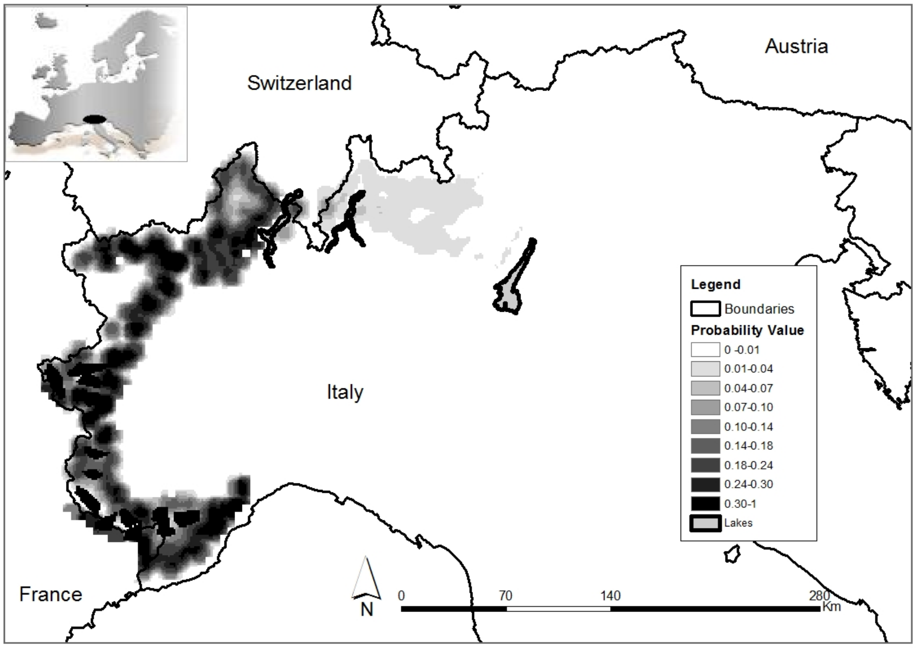
a)

b)

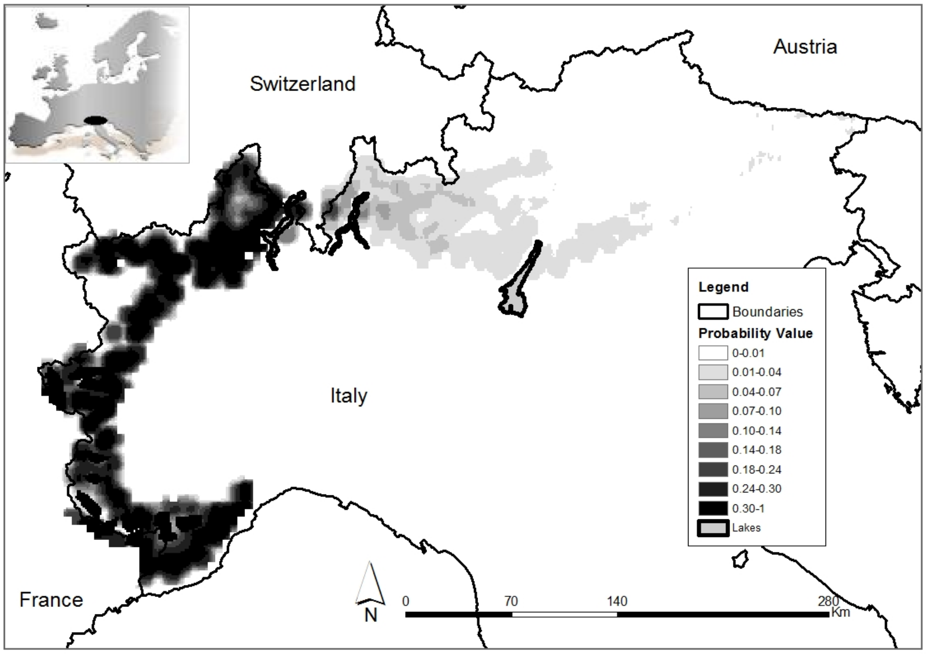
Fig. 4. Estimated (filled circles and solid lines; input data used in this paper) and simulated (open circles and dashed lines; results of IBM) mean number of wolf packs with 95% confidence intervals (a) and mean wolf population size with 95% confidence intervals (b) through 2023 based on 100 replicates simulations of the SE-IBM of the wolf recolonization process over the Italian Alps. Simulations started with known packs and wolves in 2008.


Switzerland
Austria
Italy
France
Legend
Boundaries
Probability Value
0-0.01
0.01-0.04
0.04-0.07
0.07-0.10
0.10-0.14
0.14-0.18
0.18-0.24
0.24-0.30
0.30-1
Lakes
N
0
70
140
280
Km

a)


Switzerland
Austria
Italy
France
Legend
Boundaries
Probability Value
0 -0.01
0.01-0.04
0.04-0.07
0.07-0.10
0.10-0.14
0.14-0.18
0.18-0.24
0.24-0.30
0.30-1
Lakes
N
0
70
140
280
Km

b)



c)

Fig. 5. Absolute probability maps which predict wolf pack locations in 2013 (a), 2018 (b), and 2023 (c) from the SE-IBM of the wolf recolonization process over the Italian Alps, with a starting scenario from 2008 over the next 5, 10, and 15 years, after 10000 simulations.

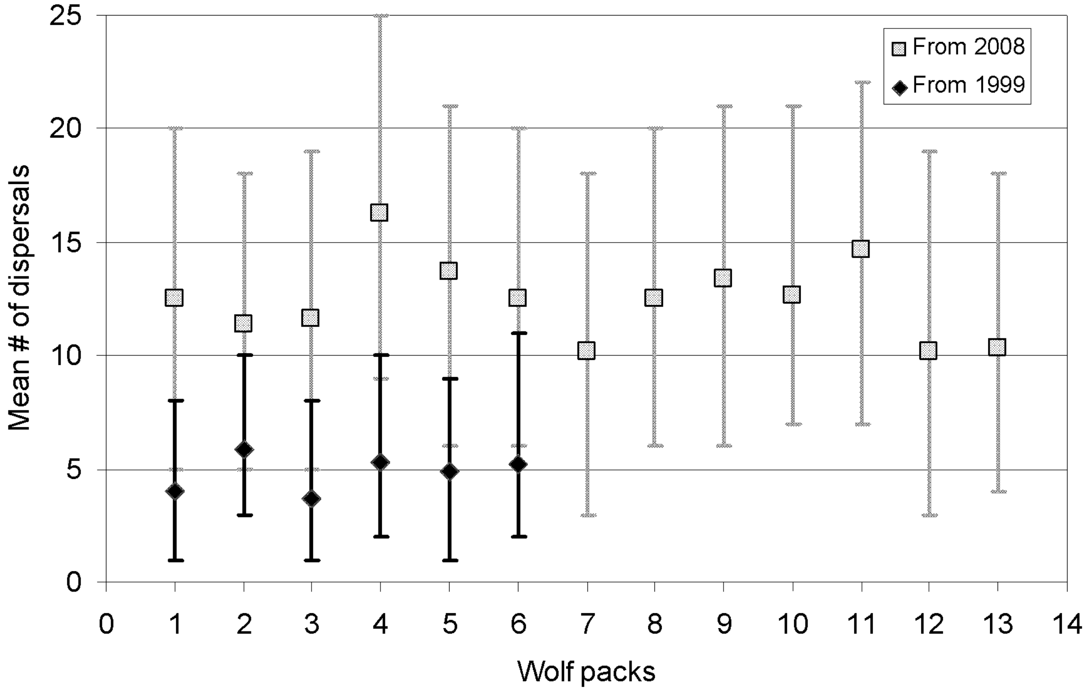


Fig. 6. Predicted mean number of wolf dispersers by pack and 95% confidence intervals from 100 replicate simulations after 10 years of simulation from the two real starting scenarios in 1999 and in 2008, using the spatially explicit, individual based model of the wolf recolonization process over the Italian Alps.