



## Research Article

## Non-invasive Integrated Sampling Design to Monitor the Wolf Population in Piemonte, Italian Alps

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### Abstract

The natural return of the wolf (*Canis lupus*) in the western Alps of Italy and France at the beginning of the 1990's, after 70 years of absence, is an important ecological and social event. The Regione Piemonte, in the course of the Progetto Lupo Piemonte, intensively monitored the wolf population over the Piemonte territory from 1999 to 2010. We estimated four main population parameters over time (wolf population size, number of packs, distribution, and effective population size) in order to follow the natural recolonization process over the Alps. An integrated sampling approach was designed to collect data to estimate simultaneously and cost-effectively these four parameters. This combined monitoring program allowed the development of specific management strategies for the mitigation of wolf-human conflicts, given the protected status of the alpine wolf population. This transboundary wolf population, which dispersed over the Alps of Italy, France, Switzerland, and now is reaching the Eastern Alps, has been considered a unique and distinct entity by the European Commission after the "Guidelines for Population Level Management Plans for large Carnivores". Therefore, it should be monitored and managed as such over the boundaries, and this cost-effective sampling approach should be considered in this framework.

## Introduction

Wolves (*Canis lupus*) were widespread in the Alps until the early 1900's when they were gradually extirpated. The last wolves were killed in

the Western Alps region during the 1920's, but wolves survived along the Apennines range of central Italy (Boitani and Ciucci, 1993). In 1971 the wolf was legally protected in Italy (Boitani, 1982), listed in the Appendix II as a strictly protected species in Europe after the Bern Convention in 1979, and in the Annexes II and IV after the Habitat Directive in 1992. Wolves began naturally recolonizing the southwestern Alps of

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Italy and France at the beginning of 1990's (Marucco, 2001; Poulle et al., 1995) through dispersal from the north Apennine wolf subpopulation (Fabbri et al., 2007). 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). 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 (Valière et al., 2003). When these semi-isolated packs and individuals appeared progressively further over the Alps, questions arose regarding wolf numbers, actual distribution, and the origin of the animals, to correctly manage this new wolf population.

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 (Dalmaso et al. 2012, this issue). The four main monitoring goals were to estimate over time: population size, number of packs, distribution, and effective population size. These four population parameters allow to properly follow demographically, spatially, and genetically the wolf population, and are the main parameters required to make management decisions. The big challenge over the years was to design and implement an effective and unique monitoring program to collect data to accurately estimate these four different parameters at the same time. Here, we define and explain the unique integrated non-invasive monitoring approach we designed to effectively follow the wolf population in Piemonte. This approach can be easily extended to the overall Alps ecosystem to follow the future expansion of the alpine wolf population, and to other situations as well. A final discussion on pitfalls and limitation of the approach will help to successfully

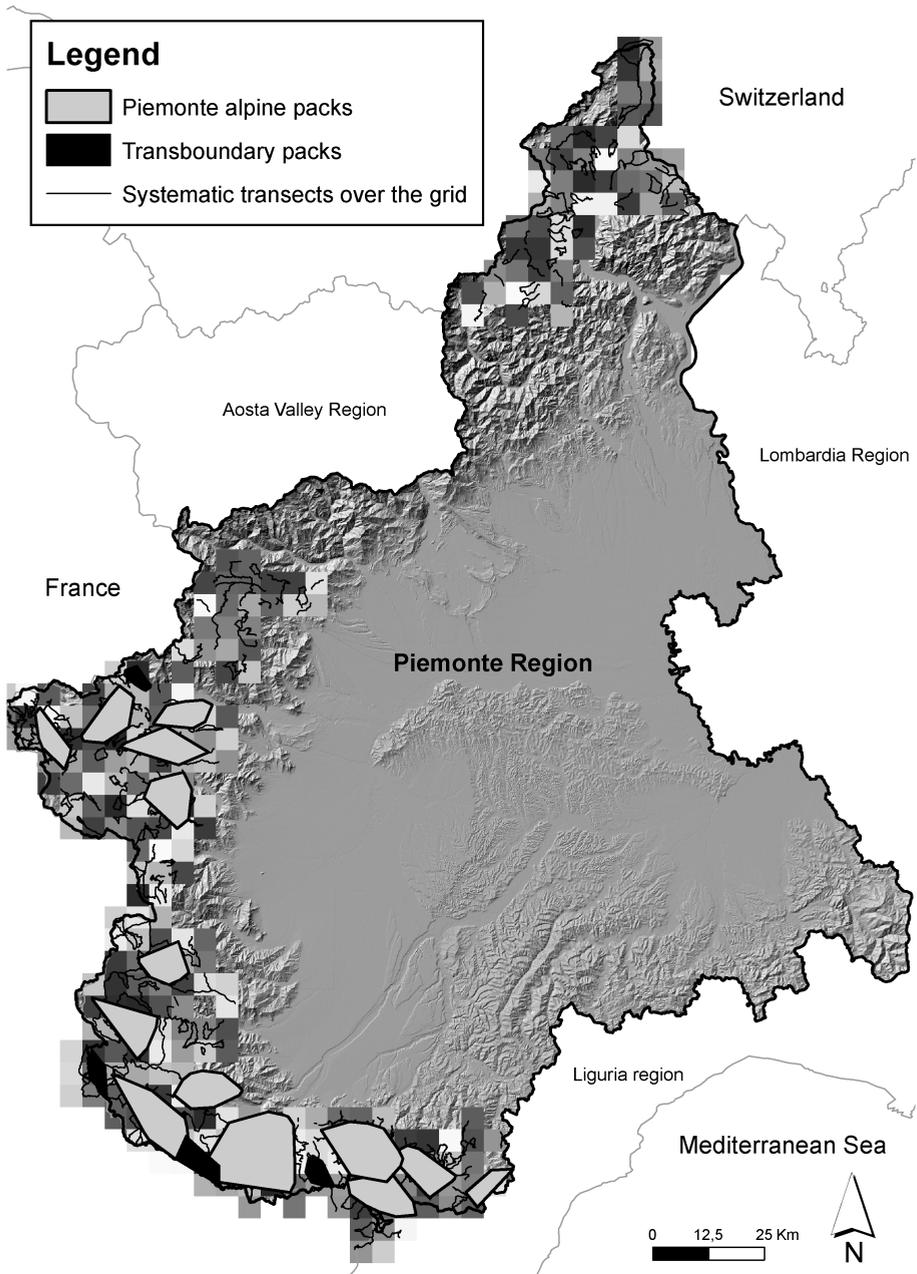
## Materials and Methods

### 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 25388 km<sup>2</sup> of which 11334 km<sup>2</sup> are in the Alps (Fig. 1). The Region consists of a mosaic of land management classes; however, part of the territory is protected in Natural and National Parks (1485 km<sup>2</sup>). The alpine area is characterized by long narrow valley bottoms surrounded by rugged mountains with elevations ranging from 450 to 4664 m a.s.l. 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 shrubland/rock areas. The snow season generally lasts from October–November through April–May.

### The integrated sampling design

The integrated sampling design was defined to simultaneously collect non-invasive data on wolves which were suitable both to a capture-recapture (CR) analysis to estimate demographic parameters, and to an occupancy analysis to estimate distribution trends (Tab. 1). We defined sampling grid cells of 5 × 5 km which covered 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 available observers. Such a cell size allowed us to monitor the smallest packs and individual territories, as well as 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), particularly useful when planning to manage 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 (Fig. 1) to collect wolf signs (i.e. tracks or scats), which were travelled from 1999 to 2010 every winter season (November–April). 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 monthly a maximum of 389 transects over the study area for a total of 3115.7 km; mean transect length was 8.0 ± 4.7 km. Transects



**Figure 1** – Sampling grid cells of 5×5 km which covered the majority of the Alps mountain range of the Piemonte Region, Italy, and transects used to collect wolf signs. Over the grids and transects, minimum territories of wolf packs of winter 2009-2010 are reported.

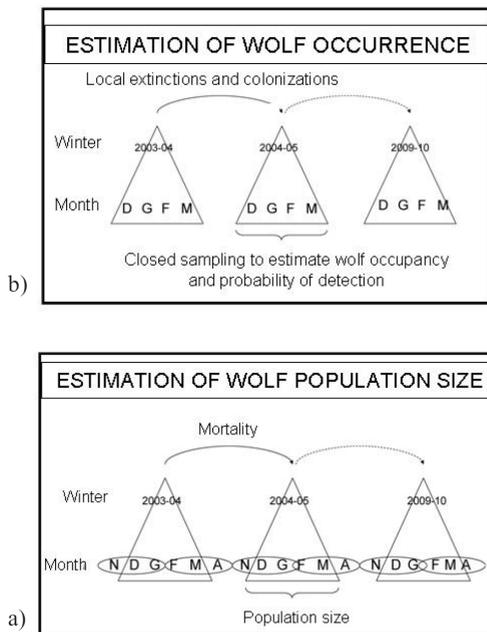
**Table 1** – The integrated sampling design defines a collection of wolf signs which allows both Capture-Recapture (CR) and Occupancy analysis, which have different goals and sampling units.

	<b>CR analysis</b>	<b>Occupancy analysis</b>
Goal	Population size and survival	Occupancy rates
Sampling unit	Individual (i.e. genotype)	Site (i.e. grid cell)
Period of study	1999–2010	2003–2010

were travelled with the objective of finding wolf travel routes, in order to follow them and collect wolf data. We conducted winter ski- or snowshoe-based surveys with several crews of 5-15 observers (generally one observer per transect) to search for wolf tracks and scats, travelling systematically every transect of the study area each winter month. Observers (the majority was personnel from the Park Service and the Forest Service) were prepared before every winter season with an intensive field and theoretical course to increase consistency in data collection. When we found wolf tracks, we followed them to collect every scat along the wolf travel route, and estimated each pack size as the maximum number of wolves traveling together that winter, as recommended by Mech (1982).

We defined an open CR sampling design where each transect of the study area was covered multiple times (1-6) during each of two winter sampling sessions: an early winter session (EW) from November to January and a late winter session (LW) from February to April (Fig.2a), to estimate population size and mortality rate using a genetic CR approach (MacKenzie et al., 2003) where each cell of the study area was surveyed at least once per month, and the overall survey was repeated four times each winter (b), to estimate occupancy rates and distribution (Marucco et al., 2009). 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 non-randomly selected a subsample 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 (Marucco et al., 2009). Genetic analysis was conducted by the USFWS-RMRS Carnivore Genetic Lab, Missoula. 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 in each capture session. Details of the genetic and CR analysis are described in Marucco et al. (2009).



**Figure 2** – Open capture-recapture (CR) sampling design where each transect of the study area was covered multiple times (1-6) during each of two winter sampling sessions: an early winter session (EW) from November to January and a late winter session (LW) from February to April (a), to estimate population size and mortality rate using a genetic CR approach. Multi-season occupancy design (MacKenzie et al., 2003) where each cell of the study area was surveyed at least once per month, and the overall survey was repeated four times each winter (b), to estimate occupancy rates and distribution (Marucco, 2009).

At the same time and with the same field design, we defined a multi season occupancy design (MacKenzie et al., 2003) 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 (Fig. 2b), to estimate occupancy rates and distribution (Marucco, 2009). This sampling design fits into the open CR sampling design, allowing just one sampling effort, which fulfilled both goals of having structured data to estimate both demographic, genetic and distribution parameters (Tab. 1). Therefore, within the occupancy analysis we defined 4 primary periods: December, January, February, and March (Fig. 2b). The sec-

ordinary periods consisted of each winter season. For the purpose of the occupancy analysis, we considered every wolf scat found along the transect and marked every wolf track encountered. We considered a cell occupied if we detected at least one confirmed wolf sign (i.e. a track or a scat) at one of the transects in the cell in a month. Details of the occupancy analysis are described in Marucco et al. (2010).

## Population parameters estimation

Genetic analysis on scat and tissue samples provided individual genotypes which allowed to conduct parental analysis and reconstruct each pack pedigree, allowing to estimate the number of packs, the minimum number of wolves per pack, the per season counts of simultaneous genotypes, and pack minimum territory size. A pack was considered when at least one male and one female were documented holding a stable territory for at least two consecutive winters or if breeding evidence was documented. We defined transboundary packs when pack minimum territory size was divided within the French and Italian territories, with a greater proportion on the French side. Genetic analysis was also used to estimate effective population size over time, a measure of the level of heterozygosity of the population which indicates the genetic status of the population.

Marucco et al. (2009) used the open population Cormack-Jolly-Seber (CJS) model, which estimated apparent survival ( $\Phi$ ) and recapture rates ( $p$ ) of wolves, and used the estimates of recapture rates from the best CJS model to derive estimates of population size using a Horvitz-Thompson-type estimator ( $t$ ) and its approximate variance. Distribution of wolf signs and pack minimum territory size were also compared to the findings of Marucco (2009) which used multi-season occupancy models developed by MacKenzie et al. (2003) to estimate the probability of wolf occupancy of a cell, the probability of extinction and colonization, and the probability of detection as functions of independent covariates to have accurate estimates on distribution trends. We also evaluated the rate of increase over the years in the total area occupied by wolves considering the  $5 \times 5$  grid cell, which better represented the distribution over our study area (Fiorentino, 2009).

## Results

### The integrated sampling design

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 and the average number of people/survey/area ranged from 2 to 15. We followed a total of 7165.5 km of wolf travel routes and collected 10312 wolf scats over the 22 sampling sessions (11 early and 11 late winter sessions). We genetically analyzed 3762 samples and successfully genotyped 1502 scats for a 40% success rate. We identified 258 unique individual genotypes from the 1502 scats that provided successful results: 24% of the individuals were captured only once ( $n = 62$ ), and of the 76% that were captured more than once, two individuals were recaptured up to 60 times over 10 years. Wolves were present and detected on average at 32% of the 265 grid cells over the years. Not all grid cells were visited at every survey; an average of 85.6% of the sites were visited per month. In 44% of the visited sites we did not find any wolf sign, and in the remaining 56% we detected wolf signs at least in one year. In particular, of those sites with positive detection histories, 34 sites had detection in only 1 year (24%), 31 sites had detection in 2 years (21%), 80 sites had detection in  $\geq 3$  years (55%).

### Population parameters estimation

The number of wolf packs increased over the years from 1 to 18 (Fig. 3) and the minimum population size documented by combining the minimum number of genotypes detected and the information from the snow tracking surveys also increased (Fig. 4). The minimum population size from 1999 to 2010 in the early winter increased from 20 to 61, and in the late winter from 17 to 52 (Fig. 4). In general, a lower estimate is present in the late winter (Fig. 4), and a non-continuous trend is detected over time, with a lower estimate in winter 2008-2009 (Fig. 4). The mean rates of population size increase were  $1.14 \pm 0.20$  and  $1.14 \pm 0.23$  in early and late winter, respectively. The minimum mean pack size is larger in the early winter ( $3.9 \pm 0.8$ )

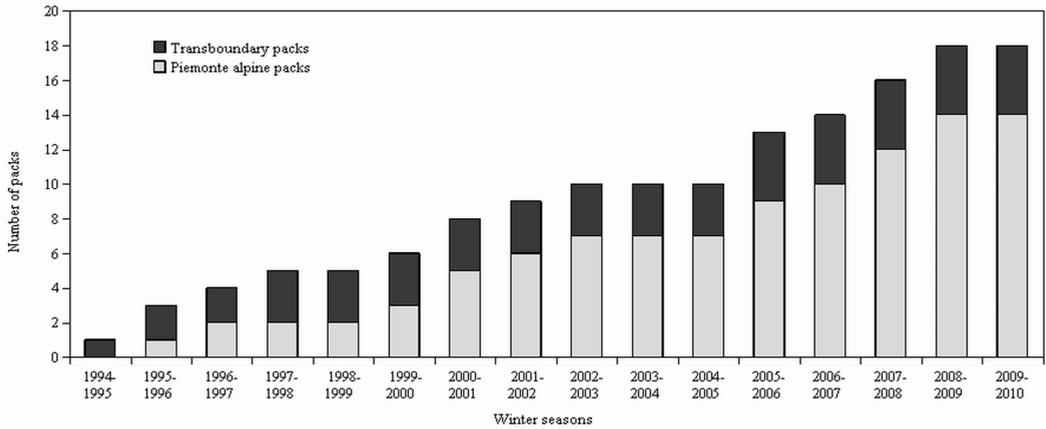


Figure 3 – Trend in the number of packs documented over the alpine area of the Piemonte Region from 1994 to 2010.

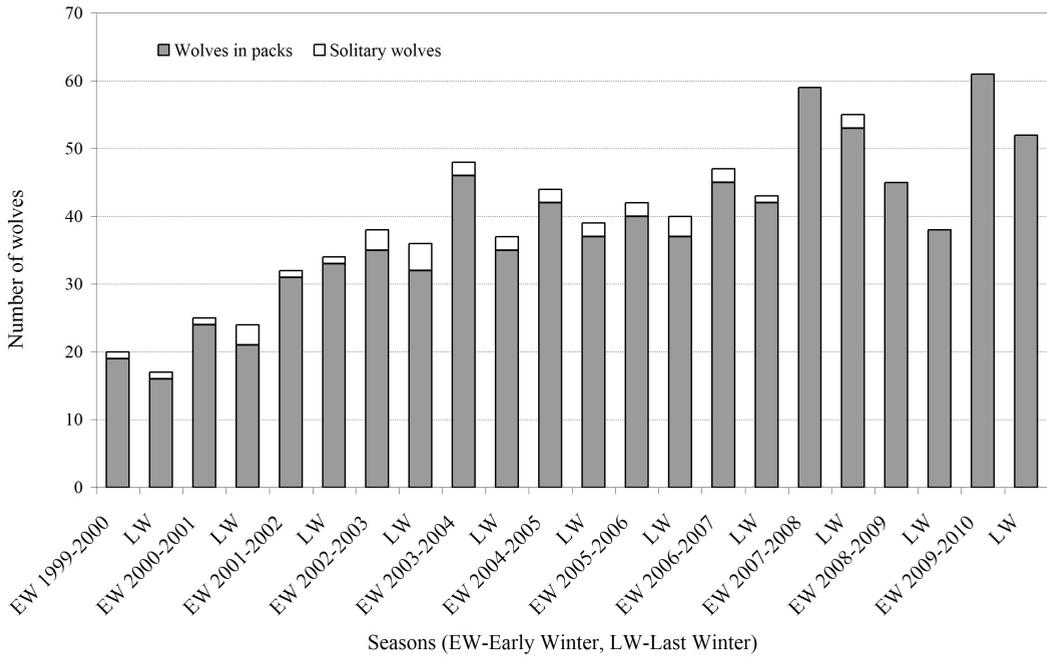


Figure 4 – Trend in the minimum number of wolves (solitary and belonging to packs) documented through snow-tracking and genetic data in early and late winters in the alpine area of the Piemonte Region from 1999 to 2010.

than in late winter ( $3.4 \pm 0.4$ ). We detected a positive trend in wolf abundance regardless of the methods used, even though we estimated on average 36.2% (SD = 13-6%) fewer wolves each season with snow-tracking data as we did by CR modeling (Marucco et al., 2009). The distribution of packs increased over the Italian Alps moving northward (Fig. 1), and we documented an average  $1.20 \pm 0.28$  annual rate of increase in the total area occupied over the years, considering the  $5 \times 5$  grid cells, also if Marucco (2009) detected a constant occupancy rate over 5 years indicating the slow recolonization process over a shorter timeframe.

## Discussion

We successfully implemented a new, integrated, non-invasive, and large-scale study design to simultaneously assess distribution, genetic, and demographic parameters of a wolf population in the Alps of western Europe. These parameters are fundamental to correctly manage and implement conservation actions of this endangered species. We have documented through these simultaneous approaches that the wolf population increased over the 11 years of the study, as did the number of packs observed. All methods we used supported this trend, though we estimated fewer wolves every season with snow-tracking data than by CR modeling as documented by Marucco et al. (2009). Packs are the primary social units of a wolf population, but the population is composed of lone wolves, typically dispersers, too (Fuller et al., 2003). Genetic CR modeling is more likely to identify these individuals than is snow-tracking counts. Dispersers and lone wolves are counted in the genotype estimate and are characterized by low recapture rates, which indirectly increases the overall CR population size estimate (Marucco et al., 2009). CR estimates are more accurate than minimum counts (Lebreton et al., 1992), and more likely to identify solitary wolves, which can be particularly abundant especially in newly recolonized areas, such as the Alps. In fact, the majority of the population in our study is composed of young wolves, which is typical of wolf recolonization areas with plentiful suitable habitat

(Fuller et al., 2003). However, genetic CR population size estimates should always be corroborated with estimates of the number of packs to effectively quantify the trends over time, which are largely dependent on the development of new territorial packs. In fact, population size estimates are more fluctuating than number of packs estimates, likely because the number of wolves in packs can largely vary over the years depending on local mortality or higher intra-pack competition for food, while the number of packs are more robust to these local changes. Lower abundance estimates occur during LW, as expected based on the life cycle of wolves; hence, the late winter estimate is likely more conservative. Similar results have been found in North American wolf populations (Fuller et al., 2003; Pletscher et al., 1997). Distribution rate of increase showed the same trend as population size and pack trends.

The integrated approach we designed, which is completely based on non-invasive techniques, is an optimal approach which allows the use of the genetic dataset in a modeling framework. Genetic techniques are a new important tool, now largely used for large scale population monitoring (Schwartz et al., 2007) for a variety of species and locations. However, specific sources of error are present using a non-invasive dataset (e.g. Creel et al. 2003), and limitations in the use of the data are present if a robust design is not implemented in the data collection. We demonstrated how important it is to collect structured data to produce a dataset which is possible to analyze with newly sophisticated modeling techniques, such as occupancy or CR applications, to produce robust estimates that need to be corroborated by traditional ones.

## Lessons learned

- It is fundamental to clearly define the objectives of the monitoring and the study design, as well as the sampling design prior to collecting non-invasive samples. This includes taking into account the assumptions of the models to be used, and the number of samples expected to be analyzed in the genetic lab to reach the desired level of recaptures and information,

while avoiding over-sampling and minimizing effort.

- If the goal is to monitor the wolf population in the Alps as a unique population, as indicated by Linnell and Boitani (2012, this issue), we should focus on estimating pack numbers and distribution over the years, more than population size, because a common robust CR estimate of population size is hard and expensive to obtain given the difficulties in calibrating the datasets in between different genetic labs.
- In general, the late winter population size estimate is likely more conservative and should be chosen for management purposes.
- Pitfalls should be considered in designing sampling and lab protocols to minimize errors.
- A sampling design based on collection of scats along wolf tracks increases the probability of characterizing each individual, especially young and dispersing wolves. This can increase the probability of detection, especially important when funding is limited.
- The sampling design described here should be adapted to larger study areas, especially considering a lower field effort by avoiding multiple encounters of an individual within a sampling session, or multiple transects within a cell.
- Defining wolf occurrence over the boundaries will need an assessment of standards for data mapping. 

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