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Wolf ecology in the western Alps: Analysis with non-invasive techniques

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WOLF ECOLOGY IN THE WESTERN ALPS: ANALYSIS WITH NON-INVASIVE TECHNIQUES

by

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Wolf Ecology in the Western Alps: Analysis with Non-Invasive Techniques (94 pp)

Director: Daniel H. Pletscher DHP

Abstract:

The natural return of the wolf (*Canis lupus*) in the southwestern Alps of Italy and France, far from known occupied wolf ranges in the Apennines Mountains, raised questions regarding the ecology of the animals in such a fragmented area with high human density. I conducted this study on the ecology of a wolf pack in this recently recolonized area with the use of non-invasive techniques during 3 years (1999-2002) to monitor wolf pack dynamics and wolf food habits.

My first objective was to examine wolf pack dynamics using a combination of 3 non-invasive techniques. I estimated pack size and followed the social history of individuals combining data from wolf-howling surveys during the summer, snow-tracking surveys during the winter, and genetic analysis on scat samples collected over the entire year. Although there was a consistent pack size of 5-6 individuals during each winter a high yearly turn-over of individuals within the pack occurred. I could not distinguish between dispersal and mortality, but reproduction was documented each summer.

My second objective was to examine diet selection of wolves along wolf travel routes during winter and to assess an optimal sampling design for scat collection to investigate wolf diets. Roe deer (*Capreolus capreolus*) was the main prey species for wolves in the area. Diet components differed between winters; relative use of red deer (*Cervus elaphus*) was greater in winter 2001, while use of wild boar (*Sus scrofa*) was greater in winter 2002 than during other winters. I evaluated possible biases in the scat collection method and determined an optimal sampling design using Monte Carlo simulations. I examined changes in resolving power with respect to effort expended for a range of possible sample sizes.

The optimal sampling design to monitor wolf pack dynamics and wolf food ecology through time will allow Park Service or Forest Service personnel to develop continuous monitoring protocols for future research efforts.

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ACKNOWLEDGMENTS

This study involved the cooperation of many people and organizations, and I have many to thank. Funding has come from the European Community and the Piemonte Region. I appreciate generous logistical support from the Alpi Marittime Natural Park and Alta Valle Pesio Natural Park, Italy. I owe an enormous thanks to Giuseppe Canavese for the financial organization of the project; it is hard to find a manager who supports and believes in research more than Giuseppe.

The amount of fieldwork in these 3 years of the wolf research project was extensive due to the large study area, the rugged mountains, the logistics, and the fact that we had to deal with such elusive animals. My research would have been impossible without the hard work of two wolf project researchers: Tommaso Galli and Luca Orlando. They conducted excellent work while I was attending courses at the University of Montana. I thank you for this and for the wonderful experiences we shared working with such a fascinating animal in the rugged mountains of Italy. I thank also my colleague Simone Ricci for numerous helpful discussions on wolf ecology and conservation. I thank all the research assistants who worked on the wolf project in these 3 years: Eglantine Aubin, Alessandro Barabino, Meagan Bayless, Carlalberto Cimenti, Mattia Colombo, Jessica Meredith, Sabrina Manchi, Vanessa Rauel, Paola Ramondo, and Alessandra Tropini. I thank all the park rangers of the Valle Pesio Natural Park for the fieldwork and help in many different situations, in particular Davide Sigaudo, Massimo Sciandra, Giuseppe Gerbotto, Tino Gerbotto, and Mauro Fissore. I thank also the park rangers of the Mercantour National Park in France, and Benoit Lequette, the scientific coordinator of the Park. International collaboration was fundamental for monitoring the transboundary territories of these wolves. Because of all the people mentioned above, all the field work conducted should be considered as "we". I used "I" throughout the document to be responsible for every methodological decision and analysis.

Diet analysis was conducted in the lab at the Alpi Marittime Natural Park by Luca Orlando, Tommaso Galli, Eglantine Aubin, and myself. Genetic analysis was conducted at the Istituto Nazionale della Fauna Selvatica, in Bologna, Italy. Elena Fabbri, Vittorio Lucchini, and Ettore Randi were responsible for the mtDNA and nDNA analysis. Francesco Crosetto helped with GIS images.

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My deepest thanks to my supportive family, especially my mom, who encouraged my independence and scientific curiosity, and my dad, who taught me to love my mountains and freedom. They helped me in every step of the way with love and support. I would like to thank Lucky and Yukon, my canine companions who were the most faithful research assistants and all my Italian friends who supported me in all my crazy adventures.

Finally, I wish to thank the wolves of the Alps. During the almost 4 years I spent tracking these wolves I learned to appreciate the wildlife in the Alps that few people have ever seen. They have shown me that there are still some wild areas in Europe which are important to be preserved. I feel privileged to have had the opportunity to try to understand what it is like to be a wolf in such a rugged and threatened landscape. You made me understand how I want to live for the rest of my life.

I dedicate this thesis to my Nonna Franca.

Chapter 1. Extended Introduction

BACKGROUND

Wolves (*Canis lupus*) were extirpated throughout most of Western Europe during the 20th century, yet populations remained in Spain, Portugal, and Italy (Boitani and Ciucci 1993, Petrucci-Fonseca and Promberger 1993, Vila et al. 1993). Even in these countries, wolves faced ecological conditions characterized by the reduction of their natural prey species (Promberger and Schroder 1993). Wolves have shown considerable ecological plasticity, surviving on both domestic and native prey sources (Boitani 1982). Ecological conditions in Western Europe are improving and both wild ungulate and wolf populations are increasing (Francisci and Guberti 1993). Therefore, the wolf's role in the regulation of wild ungulate populations (Fritts and Mech 1981, Ballard et al. 1987, Gasaway et al. 1992) is becoming more important in some Western European ecosystems.

Wolves were widespread in Italy 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, Figure 1). The combination of increasing ungulate populations, a decline in the local human population, and legal protection since 1971 (Boitani 1982) set the stage for wolf recovery. Dispersal and subsequent recolonization are currently occurring from wolf populations in Central and Southern Italy. The range of wolves has recently expanded northward along the Apennines Mountains and in 1992 once again reached the Western Alps (Poulle et al. 1995, Figure 1). Natural wolf recovery in these areas presents interesting ecological questions and management implications. The return of the wolf raises issues among

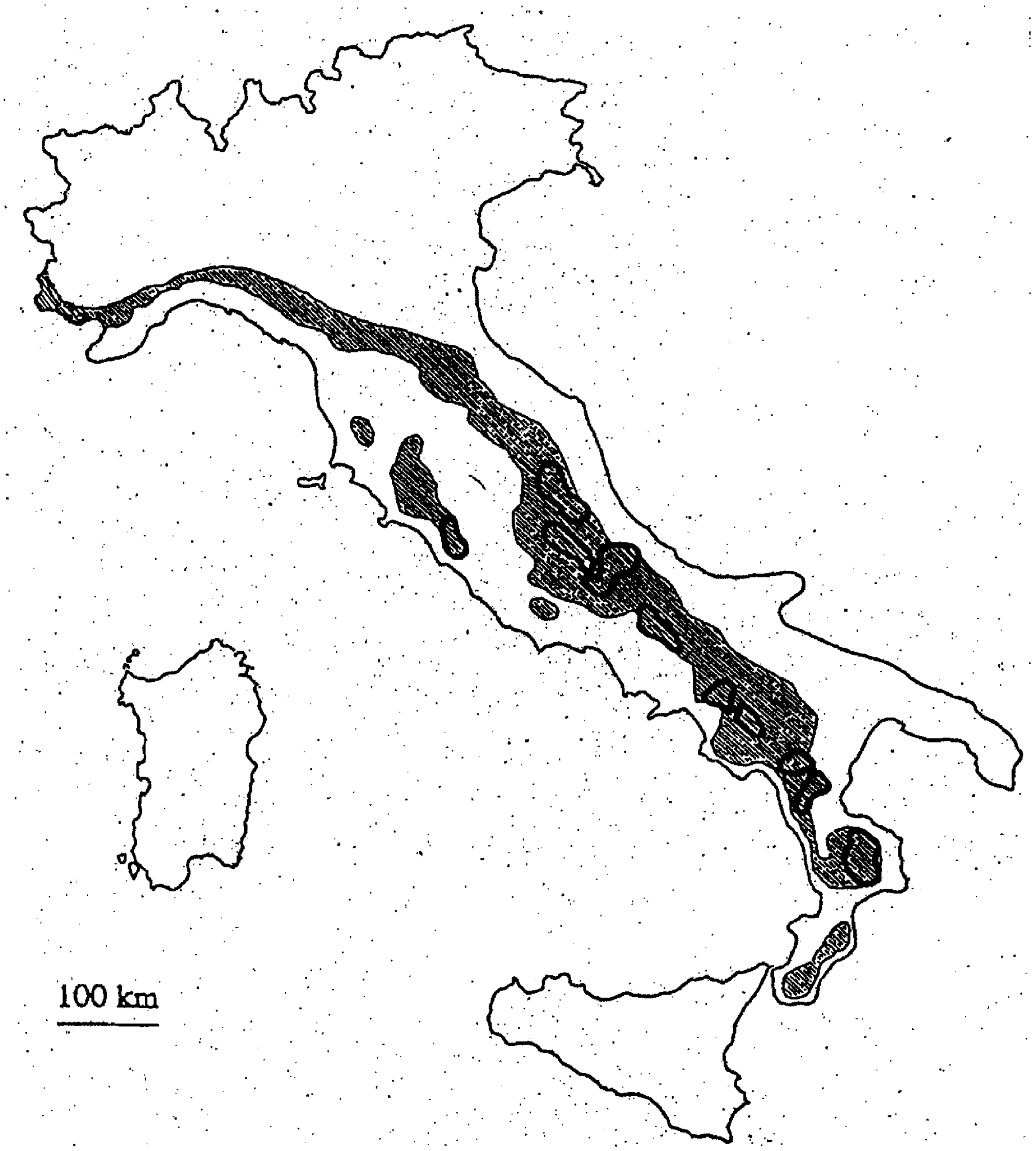


Figure 1. Wolf distribution in Italy in 1975 (dark lines, Zimen and Boitani 1975), and in 1998 (shaded area, Corsi et al. 1999).

hunters regarding competition for prey, and among shepherds concerned with losses of domestic sheep and goats. Data on food habits, wolf prey selection, and wolf pack dynamics may dispel misperceptions and thereby help direct management decisions (Litvaitis 2000).

When wolves appeared in the southwestern Alps, far from known occupied wolf ranges in the Apennines Mountains, questions arose from managers, hunters, and members of the public regarding the origin of the animals. Because of this the European Community and the Piemonte Region funded the "Interreg Wolf Project" in the Alps from 1999 to 2002 to study the wolf recolonization process. My study on the ecology of a wolf pack in a recently recolonized area in the southwestern Alps is part of this Interreg Wolf Project.

OBJECTIVES

My overall objective was to evaluate the ecology of a single wolf pack in a recently recolonized area in the southwestern Alps of Italy and France. My thesis is composed of 2 main chapters on the use of non-invasive techniques to monitor wolf pack dynamics and wolf food ecology through time:

- Chapter 2: Non-Invasive Methods to Investigate Wolf Pack Dynamics in the Western Alps
- Chapter 3: Winter Diet Selection of Wolves in the Western Alps: Optimization of a Sampling Design

My first objective was to examine wolf pack dynamics using a combination of 3 non-invasive techniques: snow-tracking surveys during the winter, wolf-howling surveys during the summer, and genetic analysis conducted on wolf scat samples collected throughout the year. I discussed the limits and the concordance of the methods, assessing an optimal sampling design to monitor the wolf population over the Alps range to follow the natural recolonization process.

My second objective was to examine wolf winter diet selection along wolf travel routes and to assess an optimal sampling design for scat collection to investigate wolf diets. This will allow Park Service or Forest Service personnel to develop continuous monitoring protocols for future research efforts.

LITERATURE CITED

Ballard, W. B., J. S. Whitman, and C. L. Gardner. 1987. Ecology of an exploited wolf population in south-central Alaska. Wildlife Monographs **98**:75-81.

Boitani, L. 1982. Wolf management in intensively used areas of Italy. Pages 158-172 in F. H. Harrington, and P. C. Paquet, editors. Wolves of the world. Perspectives of behavior, ecology and conservation. Noyes publications, Park Ridge, New Jersey.

Boitani, L., and P. Ciucci. 1993. Wolves in Italy: critical issues for their conservation. Pages 74-90 in C. Promberger, and W. Schroder, editors. Wolves in Europe. Status and perspectives. Munich Wildlife Society, Ettal, Germany.

Corsi, F., E. Duprè, and L. Boitani. 1999. A large-scale model of wolf distribution in Italy for conservation planning. Conservation Biology **13**:150-159.

Francisci, F., and V. Guberti. 1993. Recent trends of wolves in Italy as apparent from kill figures and specimens. Pages 91-102 in C. Promberger, and W. Schroder, editors. Wolves in Europe. Status and perspectives. Munich Wildlife Society, Munich.

Fritts, S. H., and L. D. Mech. 1981. Dynamics, movements, and feeding ecology of a newly protected wolf population in Northwestern Minnesota. Wildlife Monographs **80**:1-78.

Gasaway, W. C., R. D. Boertje, D. V. Grangaard, D. G. Kelleyhouse, R. O. Stephenson, and D. G. Larsen. 1992. The role of predation in limiting moose at low densities in Alaska and Yukon and implications for conservation. Wildlife Monographs **120**:1-59.

Litvaitis, J. A. 2000. Investigating food habits of terrestrial vertebrates in L. Boitani, and T. K. Fuller, editors. Research techniques in animal ecology. Controversies and consequences. Columbia University Press, New York.

Petrucci-Fonseca, F., and C. Promberger. 1993. Population trends of wolves in Portugal. Pages 110-112 in C. Promberger, and W. Schroder, editors. Wolves in Europe. Status and perspectives. Munich Wildlife Society, Ettal, Germany.

Poulle, M.-L., T. Houard, and T. Dahier. 1995. Le suivi des loups dans le Parc National du Mercantour. Bulletin mensuel de l'Office National de la Chasse 201:36-45.

Promberger, C., and W. Schroder 1993. Wolves in Europe. Status and perspectives. Munich Wildlife Society, Ettal, Germany.

Vila, C., J. Castroviejo, and V. Urios. 1993. The Iberian Wolf in Spain. Pages 104-109 in C. Promberger, and W. Schroder, editors. Wolves in Europe. Status and perspectives. Munich Wildlife Society, Ettal, Germany.

Zimen, E., and L. Boitani. 1975. Number and distribution of wolves in Italy. Saugetierkunde 40: 102-112.

Chapter 2. Non-Invasive Methods to Investigate Wolf Pack Dynamics in the Western Alps

Abstract: I investigated the dynamics of the Valle Pesio wolf (*Canis lupus*) pack for 3 years using a combination of 3 non-invasive techniques. I estimated pack size and followed the social history of individuals considering data from wolf-howling surveys during the summer, snow-tracking surveys during the winter, and genetic analysis on scat samples collected over the entire year. Single techniques do not provide a good estimate of the population size; a combination of the 3 provides the most reliable population estimation and provides evidence for the social history of individuals in the pack. Although there was a consistent pack size of 5-6 individuals during each winter a high yearly turn-over of individuals within the pack occurred. I could not distinguish between dispersal and mortality, but reproduction was documented each summer. Uncertainty concerning the relative role of dispersal and mortality highlights the need for radiotelemetry studies over a larger area.

INTRODUCTION

Large-scale studies of pack dynamics based on methods which allow monitoring the life history of individual wolves are of paramount importance for wolf conservation in the Alps. Conservation strategies for mammalian carnivores include the management of disjunct populations, where survival at a larger regional scale often depends on growth and dispersal characteristics of local populations (Haight et al. 1997). Therefore, the monitoring of individual wolf packs through at least several years may provide important information on critical components of population dynamics (Ciucci and Boitani 1999). However, intensive research and monitoring programs on local wolf packs have been limited in Italy (Boitani 1976; Ciucci 1994;

Boitani and Ciucci 1996) because the non-invasive methods used (snow-tracking and wolf-howling surveys) did not provide information on the history of individual wolves (Ciucci and Boitani 1999). Therefore, important components of population dynamics such as mortality, dispersal, reproduction, and recruitment are still almost unknown (Ciucci and Boitani 1999).

In most cases, radiotelemetry is the best available technology to determine the movement patterns and population demographics of elusive and secretive animals (Millspaugh and Marzluff 2001); however, live-trapping wolves and monitoring a representative sample of radio-collared individuals in populated countries such as Italy is not always feasible (Ciucci and Boitani 1999).

Rarity makes direct observation difficult; live-trapping is troublesome and sometimes dangerous for endangered and low density species like wolves. Additionally, capture efforts are expensive and take considerable time and effort (Kohn and Wayne 1997). Non-invasive techniques are preferred whenever they can provide the type, quality, and quantity of data needed. Genetic techniques can be used in the analysis of faeces (molecular scatology) to address taxonomic issues and demographic questions, through individual identification via 'genetic fingerprinting' (Mills et al. 2000) and sex determination with gender specific markers (Kohn and Wayne 1997).

In this study, I examined how the combination of data from conventional non-invasive techniques such as snow-tracking and wolf-howling surveys, with data from newly emerging DNA-based techniques, may provide a much more comprehensive picture of the hidden life of the elusive wolf population in the Alps. Wolf-howling and snow-tracking can document reproduction and determine the number of wolves in a pack, while newly emerging DNA-based techniques can document the individual identity of wolf pack members. I monitored wolf pack dynamics and the fate of each individual wolf in the pack for 3 years. By illustrating application of the non-invasive methods used (snow-tracking, wolf-howling, and genetic analysis on scat samples), I also considered their limitations in terms of interpretation of wolf pack dynamics, and concordance between the methods. Finally, I proposed an optimal sample collection to document wolf pack dynamics over the long term.

STUDY AREA

The study area was defined by wolf presence and is located in a mountainous region of the southwestern Alps of Italy and France (Figure 1). The area consists of about 800 km^2 and encompasses the Alta Valle Pesio e Tanaro Natural Park (67.7 km^2) and adjacent lands. The core area is characterized by long narrow valley bottoms surrounded by rugged mountains, with elevations ranging from 800-2651 m. Dense coniferous and broadleaf forests (*Abies alba* and *Fagus sylvatica* are prevalent) cover about 50% of the area, 30% consists of alpine meadows, and 20% of bushes and rocky areas. The few roads in the area are closed during winter. The annual average precipitation is 1285 mm and the snow-season generally goes from November to April. Few human settlements are in the area due to a steady and constant decline in the human population during the past 30 years. However, human density in the region remains high. The most common ungulate species in the area are chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), and red deer (*Cervus elaphus*). Populations of roe deer, chamois, and wild boar in the study area today are abundant as a consequence of reintroductions by the Park System beginning in the 1980s and of natural range expansion by ungulates throughout Italy (Mattioli et al. 1995). The study area encompassed parts of Italy and France, and each country

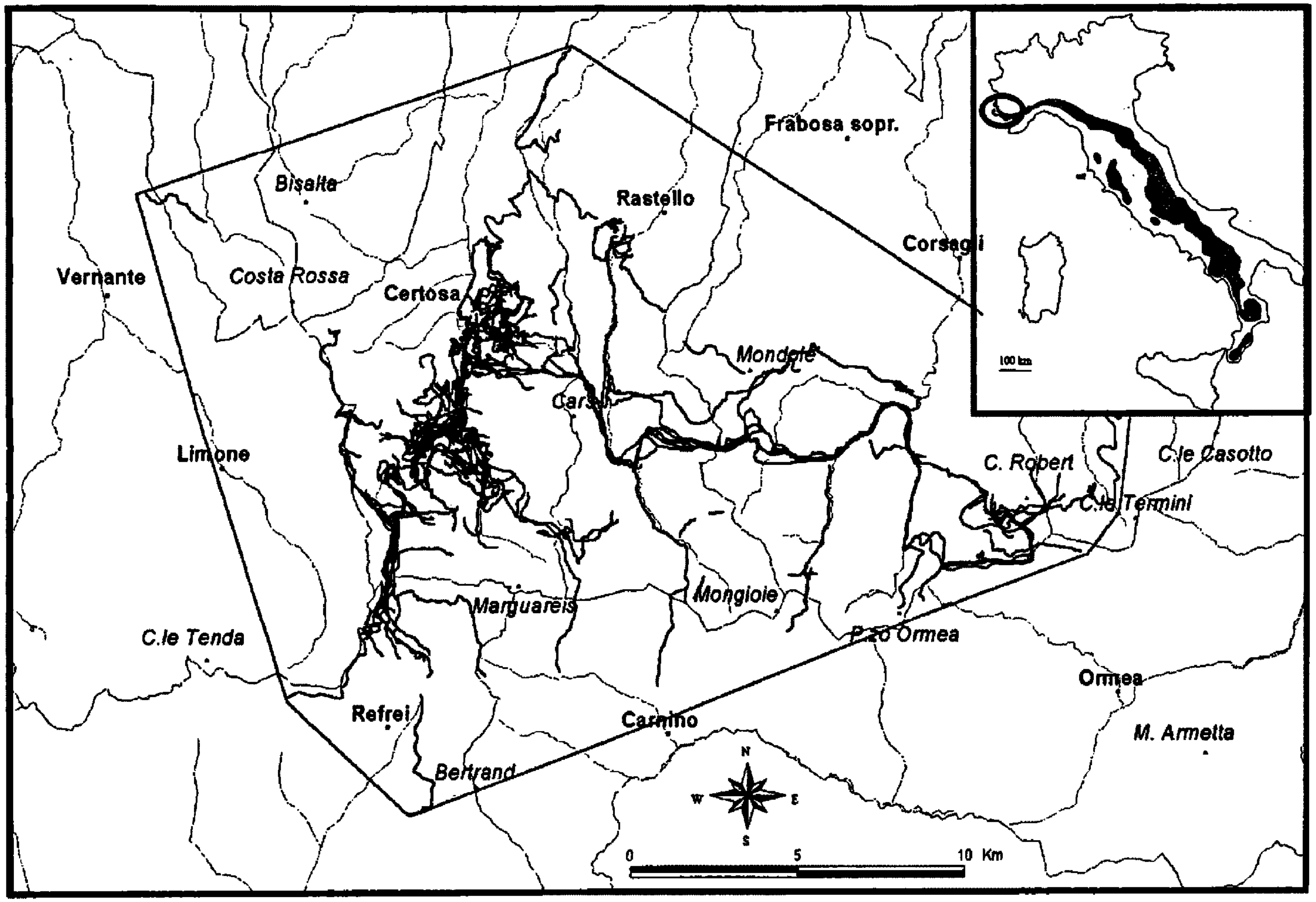


Figure 1. Study area defined by tracks of wolves of the Valle Pesio pack followed during 3 winter seasons (1999-2002) in the south-western Alps, Italy and France.

consists of a mosaic of land management classes; therefore, a unique management plan does not exist.

METHODS

I determined wolf pack dynamics through a 3-year period (1999-2002) by combining the data from snow-tracking sessions during the winter, wolf-howling surveys during the summer, and genetic results conducted on scat samples collected throughout the year.

Snow-Tracking

I evaluated wolves' exclusive presence in the area, pack size, and the presence of adjacent packs through winters using snow-tracking data (Tucker et al. 1990). One day after a snowfall, I searched for wolf tracks by travelling transects on skis or snowshoes. One day provided adequate time for tracks to be made, yet not enough time for other tracks to accumulate and make wolf tracks difficult to discern (Tucker et al. 1990). I selected transects on the basis that wolves spend most of their time on ungulate wintering areas or travelling between wintering areas (Mech 1970); therefore, I delineated ungulate winter ranges and then laid out transects between them (Tucker et al. 1990). Transects also followed trails and roads generally used by wolves for their movements (Carbyn 1974). When I found wolf tracks, I followed their travel routes, first in the opposite direction of wolf travel, and then, on subsequent days, I continued following wolf routes in both directions avoiding disturbing the wolves (Kunkel 1997). I did not follow travel routes forward on the first day to minimize my effect on wolf behavior. In this way, I travelled a wolf travel route on different days and I considered each continuous route a "snow-tracking session" (Ciucci 1994). I estimated the number of wolves when they spread out into individual trails along the travel route, and I defined the pack size in winter as the maximum number of wolves travelling together (Ciucci and Boitani 1999). I conducted surveys with a larger crew after large snowfalls to determine the exclusive presence of wolves in an area and the presence of adjacent packs in the overall study area.

Wolf-Howling

I assessed the production of pups during summers (1999-2002) by eliciting howls at rendezvous sites (Fuller and Sampson 1988, Harrington and Mech 1982). I followed Harrington and Mech (1982) specifications, repeating each howling station for 3 consecutive nights to document absence or presence of the pups in the overall study area. I recorded responses into 0, 1, or ≥2 pup categories (Ciucci and Boitani 1999), because accurate counts of > 2 pups were difficult to obtain, especially when subadults, adults, and pups joined the chorus (Harrington and Mech 1982).

Genetic Analysis

Random sampling of wolf scats was not logistically feasible. The sampling design for summer and winter 1999-2002 scat collection was different; during the winter scats were collected along wolf travel routes to identify each individual, whereas during the summer, scats were found along roads and trails, because no other collection method was feasible. Scats collected at the rendezvous sites were too old for genetic analysis. In order to minimize disturbance to the pups, the rendezvous site was not entered until October, therefore fresh scats could not be obtained.

I stored each wolf scat collected at -30°C until transferred to test tubes containing 95% ethanol. Contamination in the field may occur; therefore, I was careful with the material used for collection, and in the steps of transferring scats into ethanol.

I non-randomly selected a sub-sample of scats for the genetic analysis on the basis of field observations to maximize the probability of identifying individuals while optimizing laboratory efforts. I selected only fresh scats for analysis. I used a winter criterion of scat selection for the genetic analysis that prioritized groups of scats found along the same snow-tracking session. In this way, the probability of characterizing each individual in the pack was higher. During summer, I prioritized fresh scats for the genetic analysis to optimize the laboratory efforts (Lucchini et al. 2002). Scats were ranked as 'fresh' (1-2 days old when collected in summer), or 'old' (all other samples), based on a combination of time elapsed since the last sampling effort, scat appearance, exposure of deposition site, and weather conditions (Ciucci et al. 1997). I documented the related marking behavior of wolves for each scat collected (Vila et al. 1994).

Out of 927 scats collected from May 1999 to August 2002, 269 scats were analyzed at the I.N.F.S. (Istituto Nazionale della Fauna Selvatica) genetic laboratory in Bologna (Italy). DNA analysis procedures are detailed in Lucchini et al. (2002), where DNA samples, extracted from wolf scats, were genotyped to determine species and sex by sequencing parts of the mithocondrial DNA (mtDNA) control-region and ZFX/ZFY genes. Individual genotypes were identified by multilocus microsatellite analyses using a multiple tubes polymerase chain reaction (PCR). In particular, the lab used 6 microsatellites (CPH2, CPH8, CPH12, FH2079, FH2088, FH2096) to identify the genotypes during the first 2 years of analysis, and then used 6 other microsatellites (FH2004, FH2079, FH2088, FH2096, FH2132, FH2137) to improve analysis of the last year's samples (Table 4).

Genealogical relationship among individuals could not be determined reliably because of the low number of microsatellites analyzed (10-20 microsatellites are required to reliably determine parental relationship [Queller et al. 1993]). Therefore to estimate relationships, I used a combination of kinship analysis, evaluated with values of relatedness (*r*) using the software Kinship Version 1.2 (Goodnight and Queller 1999), exclusion considerations, and field observations.

Kinship allows estimation of the likelihood of first-order relationship (i.e. dyads represent full siblings or parent-offspring) (r=0.5 for the expected relatedness of a first-order relationship vs. r=0.0 for no relationship). In this way, Kinship identifies the most likely parents even if there are exclusions at >1 locus.

Exclusion considerations are based on the concept that microsatellite alleles are being inherited in a Mendelian fashion, and if the alleles of an offspring do not fit with a parent, that's called an 'exclusion'. One exclusion in 10 loci is often not strong evidence that it is not a parent-offspring dyad (because of genotyping errors, mutations; i.e. it does not have high power to exclude an animal as a parent). However, ≥2 exclusions is strong evidence.

Moreover, I also considered important field observations. For example, snow-tracking may identify the breeding pair or scat size and location associated with track data can identify adults vs. pups or wolves belonging to other packs.

RESULTS

I determined the Valle Pesio wolf pack dynamics through a 3-year period (1999-2002). I also documented the presence of an adjacent wolf pack, the Lugo wolf pack, and of another wolf (F22) not belonging to any pack (Figure 3).

Pack size during the winter

I followed wolf tracks for a total of 94 tracking sessions and 694.1 km. In particular, I intensively followed Valle Pesio wolves tracks for a total of 89 tracking sessions and 653.3 km during 3 winter seasons (1999-2002) (Table 1, Figure 1), and Lugo pack wolf tracks for a total of 5 tracking sessions and 40.8 km only during the last winter season (Figure 2). The Lugo wolf pack size was 5.

In the following analysis I only considered the Valle Pesio wolf pack data. The entire pack was not always together; therefore the number of wolves travelling in a group varied (Table 1).

Table 1. Winter Valle Pesio pack size in the south-western Alps, 1999-2002, determined by following wolf tracks, and number of wolves of the Valle Pesio pack followed along each tracking sessions.

			Pack size		Pack association*		
Winter	**Km. tracked**	**No. sessions**	**Early Winter**	**Late Winter**	**1 wolf**	**2 wolves**	**3-8 wolves**
1999-2000	124.7	22	5	5	17.0%	26.1%	56.9%
2000-2001	256.2	29	8	6	3.5%	14.3%	82.2%
2001-2002	272.4	38	6	4	11.1%	40.0%	48.9%
Total	653.3	89			10.2%	20.2%	69.6%

* % of km tracked per group size of the Valle Pesio wolf pack

No other territorial packs frequented the study area. Mean (±SD) pack size, during the 3-winter period, was 5.7 (±1.4) wolves, ranging from 4-8 (Table 1). Average pack sizes (±SD) were similar between October-December (early winter: 6.3 ± 1.5 wolves) and January-April (late winter: 5.0 ± 1.0 wolves) (t=1.26, 4 d.f., p=0.27).

If I considered the wolf minimum home range as the polygon connecting the outermost locations on tracking routes, the minimum territory of Valle Pesio wolf pack was 316.4 km^2 in winter 2001-2002, and the Lugo pack minimum territory was 76.7 km^2 (Figure 2).

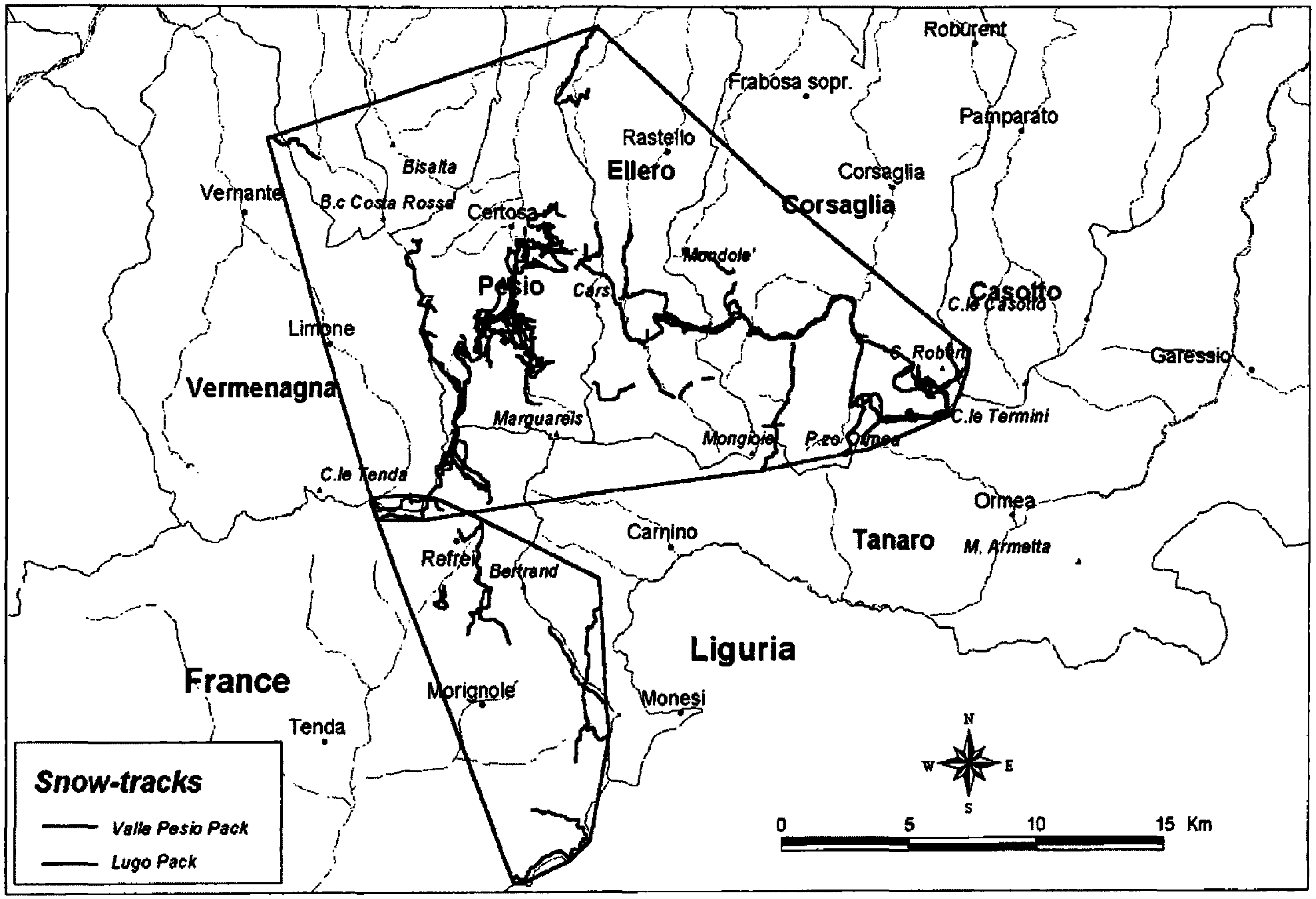


Figure 2. Valle Pesio wolf pack and Lugo wolf pack tracks followed during the 2001-2002 winter season, in the south-western Alps, Italy and France.

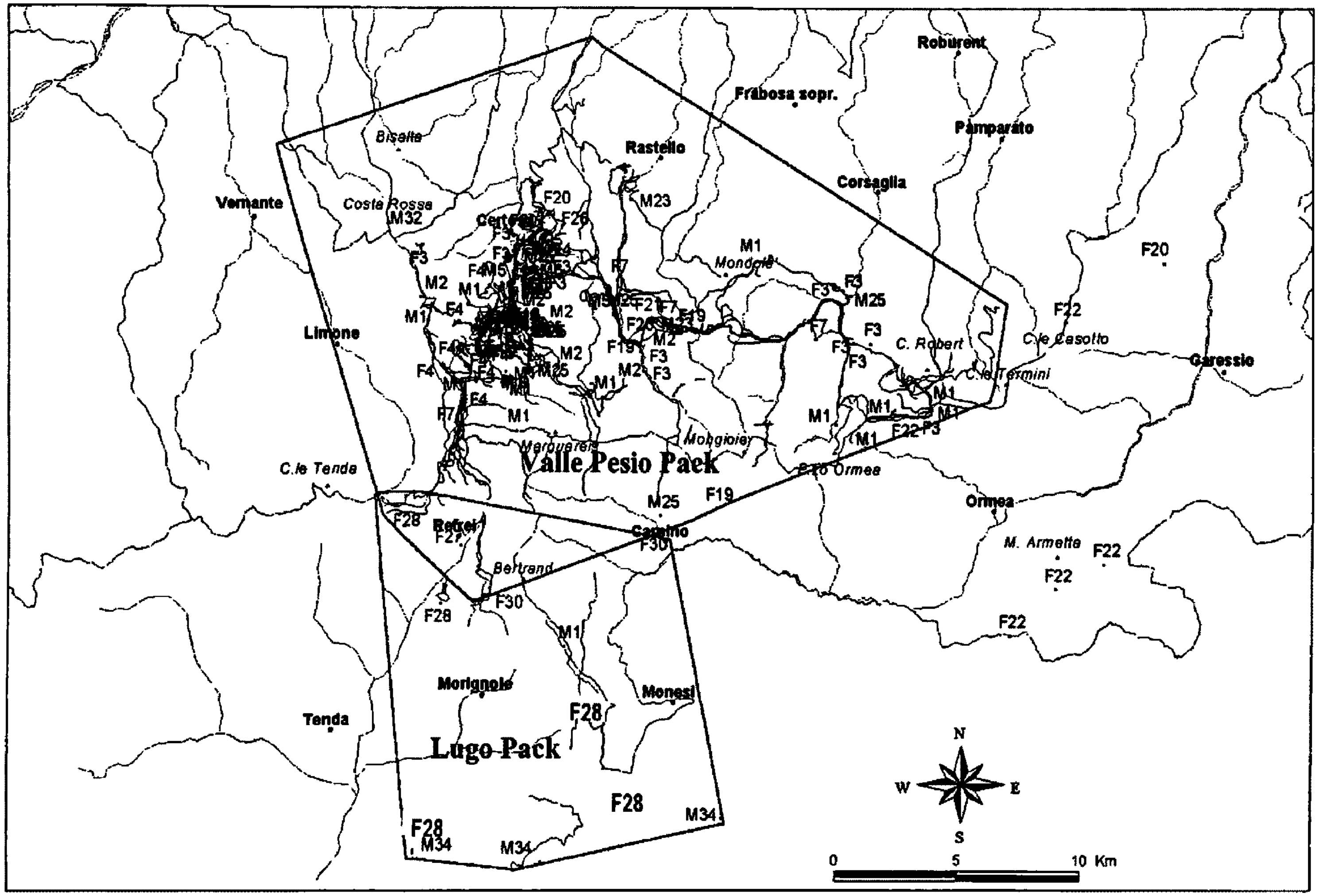


Figure 3. Wolf tracks and location of the genotypes identified from scat samples of the Valle Pesio wolf pack, the Lugo wolf pack, and F22 a solitary wolf, during 1999-2002 years, in the southwestern Alps, Italy and France.

When I considered the wolf minimum home ranges as the polygon connecting the outermost locations on tracking routes for the 3-winter season and the location of the scat samples genotyped combined, the minimum territory of Valle Pesio wolf pack was 386.7 km^2, and of Lugo pack was 159.8 km^2 (Figure 3).

At least 2 breeding adults were present in the Valle Pesio pack each winter, based on urination postures and vaginal discharges.

Reproduction

I obtained evidence of pup production in the Valle Pesio pack every summer (1999-2002). I obtained a total of 27 replies (Table 2), and in all cases ≥2 pups joined the chorus. Efforts in summer 2000 and 2002 were higher because rendezvous sites were harder to detect; the mean distance between 2 consecutive rendezvous sites was 14.3 ± 3.1 km (n=4). Wolves never used the same rendezvous site more than once from summer 1999 to summer 2002. I could exclude the presence of other rendezvous sites in the study area, since the howling efforts were distributed on the overall study area and no other replies were recorded.

Table 2. Reproduction of Valle Pesio wolf pack in the southwestern Alps, Italy and France. Number of howling sessions, related monitoring period, number of wolf replies, and estimated number of wolves (adults and pups) during summers 1999-2002.

Summer	Monitoring period	N° sessions	Replies	Estimated No. individuals	
				Adults	Pups
1999	6/7-6/10	23	9 (39.1%)	3	≥2
2000	5/7-24/9	55	6 (10.9%)	2	≥2
2001	4/7-10/9	17	7 (41.2%)	3	≥2
2002	4/7-7/9	36	5 (13.9%)	3	≥2

Social History of Individuals and Pack Dynamics

I identified 15 different genotypes in the Valle Pesio wolf pack, 4 different genotypes in the Lugo wolf pack, and a wolf, F22, not belonging to any pack (Table 3-4) (Figure 3-4). All the genotypes identified in the Valle Pesio and Lugo wolf packs showed the exclusive presence of the Italian wolf haplotype W14 in all the sequenced DNAs (Randi et al. 2000). In the following analysis I only considered the Valle Pesio wolf pack data.

The sex ratio of genotyped wolves did not differ from parity during the first 2 years of study (6F: 5M); during the last year, however, the sex ratio favored females (6F: 1M).

I examined carcasses from 2 wolf mortalities. M23, already genetically sampled from scats, died from lung disease in April 2001, and M24, not yet genetically sampled, likely died from illegal poisoning in May 2001. Both wolves were 1 year old at time of death.

I characterized 4 different genotypes during summer 1999: M1, M2, F3, and F4 (Table 4). M1, M2, and F3 were likely adults because their scats were found in June far away from the rendezvous site and were large. F4 was likely a pup because her scats were found near the rendezvous site and very small.

I genetically sampled 7 individuals during winter 1999-2000 (Table 4). M1 and F3 were the likely parents because they were found associated with vaginal discharges along snow tracks during February and March. I noticed solitary exploratory forays of M2 in March 2000, before his disappearance. F4, likely a pup, was sampled along snow tracks in association with M5, M2 and F3. F7 was sampled along snow tracks in association with M1, M2, and F3, when they hunted and rested together. M5 and F7 could have been either pups, or adults. I estimated the presence of 4 wolves and I collected 7 scats along the same wolf travel route. The genetic analysis confirmed the presence of 4 wolves: 3 of them produced 2 scats in a short period. M6 was sampled only once by himself in the core area of the Valle Pesio wolf pack.

I only sampled M1 and F3 during summer 2000 (Table 4). However, F7 was likely present in the summer, because during winter 2000-2001 she was sampled again along a track, together with M1 and F3.

At the beginning of winter 2000-2001, I monitored the presence of 8 wolves, however from January on I followed 6 wolves and excluded the presence of other wolves, through simultaneous surveys on the overall study area. I identified 4 new genotypes: F19, F20, F21, and M23, some or all of them likely pups. I can exclude the presence in the area of M2, F4, M5, and M6, because through snow-tracking surveys I can exclude the presence in the pack of 12 wolves: 8 was the highest number of wolves at the beginning of the winter, and they were M1, F3, F7, F19, F20, F21, M23, and M24.

M1 was not present during winter 2001-2002. The pack was composed of 6 females: F3 and F7 adults, F19 and F20 likely yearlings, and two new individuals, F26 and F31. M25, an immigrant, genetically different from the pack, joined the pack during the winter, and during February and March, M25 and F3 were found closely associated along snow tracks (n=9) with vaginal discharges. I noticed solitary exploratory forays of F20 in late April 2002, 30 km away from the core area of the pack (Figure 3). I excluded the presence in the area of M1, and F21. Therefore, out of 15 wolves monitored during 3 years of study, 2 died (M23 and M24) and 6 were not present at the end of the study period (M1, M2, F4, M5, M6, and F21).

		Microsatellites loci																						
Genotype	N	cph2	cph2	cph8	cph8	2088	2088	cph12	cph12	2079	2079	2096	2096	2004	2004	2079	2079	2088	2088	2096	2096	2132	2132	2137	2137
M1	17	95	103	207	211	117	125	193	193	263	275	101	101	110	110	248	260	117	125	96	96	236	236	154	178
M2	12	95	103	199	207	117	125	193	193	263	275	101	101	110	110	248	260	117	125	96	96	236	236	174	178
F3	27	103	103	207	211	117	125	193	205	263	275	97	101	110	176	248	260	117	125	92	96	236	236	154	178
F4	11	103	103	211	211	125	125	193	193	263	275	101	101	110	110	260	260	125	125	96	96	236	236	178	178
M5	2	103	103	207	211	125	125	193	193	275	275	101	101	110	110	260	260	125	125	96	96	236	236	154	178
M6	1	103	103	207	211	117	117	193	193	263	275	97	101	110	176	248	260	117	117	92	96	236	236	154	154
F7	6	95	103	207	207	125	125	193	205	263	263	97	101	110	176	248	248	125	125	92	96	236	236	154	154
F19	9	103	103	207	211	117	125	193	193	275	275	97	101	110	176	260	260	117	125	92	96	236	236	178	178
F20	5	103	103	207	211	117	125	193	193	263	275	101	101	110	110	248	260	117	125	96	96	236	236	154	178
F21	1	95	103	199	207	125	125	193	205	263	263	97	101	110	176	248	248	125	125	92	96	236	236	154	160
F22	6	103	103	199	199	117	125	193	193	275	275	101	101	110	164	260	260	117	125	96	96	236	236	168	174
M23	2+1T*	95	103	207	211	117	125	193	205	263	275	101	101	110	110	248	260	117	125	96	96	236	236	154	154
M24	1 T*	95	103	207	211	125	125	193	205	275	275	101	101	106	164	260	260	125	125	96	96	236	286	174	178
M25	13													106	110	248	260	93	125	92	92	282	286	168	174
F26	1													110	110	248	248	117	117	96	96	236	236	154	154
F27	1													176	176	248	248	117	125	92	92	236	286	154	178
F28	5													110	176	248	260	117	125	92	96	236	286	174	178
F30	1													110	176	248	260	117	117	92	96	236	286	174	174
F31	1													110	110	260	260	117	125	96	96	236	236	178	178
M34	3													110	176	248	260	117	117	92	96	286	286	154	160

Table 3. Distinct individual genotypes which were identified by microsatellite analyses (using 12 loci for the first 13 genotypes, and 6 for the others) among 124 wolf scat samples and 2 tissue samples (T*) collected in the south-western Alps, Italy and France. Genotypes are indicated by the estimated molecular weight of the 2 alleles at each locus. Each genotype was repeatedly observed in a variable number of samples (N).

	year	Summer 1999					Winter 99/00						Summer 2000						Winter 00/01						Summer 2001						Winter 01/02					
	month	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4
Valle Pesio Pack	M1 (n=17)		●		●	●●			●●●●			●			●●	●●●●						●					●									
	M2 (n=12)	●		●	●●	●●		●●	●●●		●																									
	F3 (n=27)	●	●					●●	●●	●		●		●●	●						●		●●								●	●●●	●●	●●●	●●●	●
	F4 (n=11)		●●	●●		●●●●●	●	●																												
	M5 (n=2)						●	●																												
	M6 (n=1)						●																													
	F7 (n=6)								●●										●●		●										●					
	F19 (n=9)																		●			●		●			●					●	●●●			●
	F20 (n=5)																				●	●●									●					●
	F21 (n=1)																		●																	
	M23 (n=3)																		●		●			●												
	M24 (n=1)																							●												
	M25 (n=13)																														●	●	●●●	●●●●	●●	●●
	F26 (n=1)																															●				
	F31 (n=1)																														●					
	F22 (n=6)																				●	●●●	●			●										
Lugo Pack	F27 (n=1)																														●					
	F28 (n=5)										●													●●								●		●		
	F30 (n=1)																															●				
	M34 (n=3)																							●●●												

Table 4. Genotypes characterized in the Valle Pesio and Lugo pack from summer 1999 to winter 2001-2002, in the south-western Alps, Italy and France. Every dot (•) is a scat sample; M = male wolf and F = female wolf. F22 is a solitary wolf.

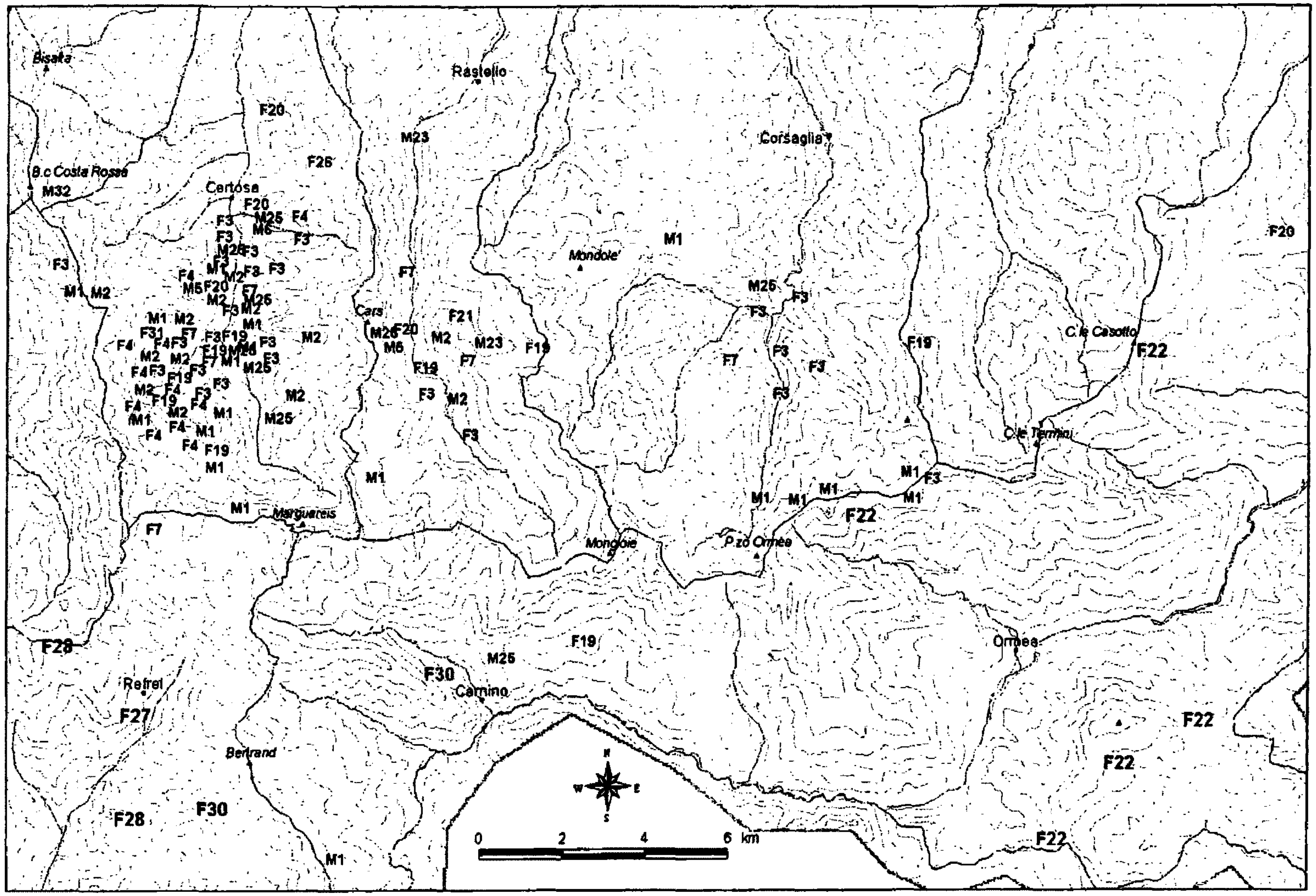


Figure 4. Location of the genotypes identified from scat samples from summer 1999 to winter 2002 in the south-western Alps, Italy and France.

Relatedness

In the Valle Pesio wolf pack I identified highly significant relationships ($p<0.001$) between wolves M1 and M2, and between F4 and M5. M1 was also related to F3, F4, and M5 ($p<0.01$). F3 was related to M6 ($p<0.01$). In the second and third years, I identified highly significant relationships ($p<0.001$) between wolves M1, M23, and F20. F3 was related to F19, F20, M23, F26, and F31 ($p>0.01$). M1 and F3, based on exclusion considerations (Table 3) and field observations, were the likely parents of F4, M5, and M6 during summer 1999; of F19, F20, M23, during summer 2000; and of F26 and F31, during summer 2001. These genotypes (M1, M2, F3, F4, M5, M6, F19, F20, M23, F26, and F31) should belong to the same pack (Table 3-4). F22, M25, M24, and F21, were not closely related to any individual in the Valle Pesio wolf pack.

The adjacent Lugo wolf pack

The Lugo wolf pack during winter 2001-2002 was composed of 5 wolves; 3 of these were genotypically identified: F27, F28, F30 (Table 4) (Figure 3-4). F28 and M34 were already present in the Lugo wolf pack area in winter 1999-2000, but only from winter 2000-2001 I did reliably document the presence of the Lugo wolf pack through snow-tracking data. Field observations and genetic data were insufficient to document the social history of these wolves. However, snow-tracking surveys documented the simultaneous presence of the 2 packs, and wolves genetically sampled within each area were more related to each other than wolves between the two territories, and were therefore subdivided into two distinct groups of relatedness (Figure 4), mostly concordant with sampling location and snow-tracking data (Figure

3). The 2 packs have a boundary area of overlap (Figure 3). Another wolf, F22, was not genetically related to other packs, and was snow-tracked by herself in areas outside the territories of other packs, or adjacent to their edges (Figure 3-4).

DISCUSSION

Limits and Pitfalls of the Non-Invasive Methods - Optimal Sampling Design for Pack Dynamics Evaluation

I used non-invasive techniques in a 3-year study on the dynamics of the Valle Pesio wolf pack. Non-invasive techniques are non-intrusive, non-destructive, and compatible with the endangered status of the wolf in most countries (Ciucci 1994). They are easy to apply, and large samples can be collected (Litvaitis 2000). I estimated pack size and dynamics considering data from wolf-howling surveys during the summer (Harrington and Mech 1982), snow-tracking surveys during the winter (Tucker et al. 1990), and genetic analysis of scat samples collected over the year (Kohn and Wayne 1997). Ciucci and Boitani (1999) followed the dynamics of a wolf pack in the Northern Apennines (Italy) for 9-years only by snow-tracking and wolf-howling surveys. They could record occurrence, numbers, and fluctuations of wolves in the area, without individually marking the members of the pack. However, they couldn't document in detail the social history of the wolf pack. In my study, molecular genetics in combination with snow-tracking and wolf-howling allowed me to follow the history of individual wolves over a 3-year period. However, the non-invasive methods used don't allow me to understand the complexity of the process of population changes. Radiotelemetry is needed to investigate dispersal, mortality, and movement patterns (Mech 1977, Millspaugh and Marzluff 2001).

The wolf-howling technique (Harrington and Mech 1982) allowed documentation of reproduction in the Valle Pesio pack every summer. The technique was also helpful to document the presence of different packs, if different rendezvous sites were monitored simultaneously. This is feasible in terms of effort if other supportive field work is conducted (snow-tracking in the winter and/or scat trails to find wolf signs); in this way howling efforts are directed in key areas and the field work is optimized. Monitoring large areas for wolf presence-absence data only using wolf-howling surveys in periods other than summer is not only highly time consuming, but also not reliable (Ciucci 1994). A test with a known population of wolves in Minnesota found that this method gave a poor estimate of population size, with wide confidence intervals (Fuller and Sampson 1988).

The snow-tracking technique (Tucker et al. 1990, Linnell et al. 1998) was optimal to document the number of the individuals in the pack and the presence of adjacent packs, if applied daily throughout the winter, using a large crew conducting simultaneous surveys on the overall area. The use of sporadic surveys, without backtracking the wolf tracks, should be limited to the evaluation of presence-absence of the species.

The recently developed molecular genetic techniques for the analysis of faeces (Kohn and Wayne 1997) were extremely important for assessing demography and social history of individuals when used in conjunction with snow-tracking and wolf-howling. Similar to other methods, genetic techniques have limits and pitfalls related to genotyping errors (false alleles, allelic drop outs, and contaminations - Taberlet et al. 1996, Gagneux et al. 1997, Taberlet et al. 1999), the shadow effect (Woods et al. 1999, Mills et al. 2000), and scat sampling methods.

Important sampling issues on wolf scat collection and subsequent selection for genetic studies must be considered in relation to the objectives of the study. How scats are sampled in the field, how they are subsequently selected for genetic analysis, and the extraction rate of the genetic lab, are all important issues which can limit the objectives of the study.

To estimate population size using genetic analysis of scat samples, Kohn and Wayne (1997) suggested that the relationship of faeces collected to new multilocus genotypes should define a curve whose asymptote represents the census population size. This applies only when genetic analysis is available with a large sample of scats. Given a low extraction rate by the genetic labs because of poor DNA quantity and quality typically found in scats (Taberlet et al. 1996), obtaining sufficient data is extremely costly. Using a non-random sampling design, wolf-marking behavior can also introduce bias, and it is likely that the curve will never reach the asymptote. The alpha pair is predominantly responsible for territorial marking in wolves (White et al. 1996).

Kohn and Wayne (1999) suggested that the approximate dimensions of territories could potentially be documented by the distribution and density of genotypes found in faeces. This was not possible in my study, due to the design of the scat sample collection and the low sample size. I sampled scats along the same travel route to characterize each individual during the winter; therefore these samples were not spatially independent. I was able to better estimate of the winter home range size for the Valle Pesio wolf pack using the snow-tracking data (Figure 1-2). However, snow-tracking data would not have indicated the dynamics of territory overlap of the 2 packs, which was elucidated by the genetic analysis. Wolves' territories overlapped, allowing them to be used by two different groups of wolves at different times (White et al. 1996) (Figure 3). Only a radio-tracking approach allows an accurate estimation of the real home range (Millspaugh and Marzluff 2001).

The goal of my study was to follow the Valle Pesio pack dynamics, and to identify each individual in the pack. Winter scats collected along wolf travel routes, those more likely to be important in evaluation of pack dynamics, were prioritized. The selection of a scat sample for genetic analysis in summer was different due to lack of related information from snow-tracking. Of the scat samples collected, cost limitations reduced the number of scats that were analyzed. Genetic results cannot be obtained from every scat sample analyzed, due to a low extraction rate in the lab. Although the collection and the selection of the scat sample were directed to identify each individual in the pack, not every sample provided genetic information due to DNA degradation (Taberlet et al. 1996) and this limited the information acquired.

Therefore, genetic results by themselves are limited. Due to the small and non-random sample, the absence of an individual from the pack could not be confirmed considering only the genetic results (Table 3). Snow-tracking data can help determine the number of wolves in the area during each winter. Tracks of 8 wolves were followed during winter 2000-2001 in the Valle Pesio wolf pack (the highest number of wolves in the winter), and knowing the genotype of each individual, I could exclude the presence of other wolves.

The fact that a wolf was not genetically sampled in the summer did not mean that it was not present at that time. Sampling during the summer did not indicate the presence of pups, except for F4 during summer 1999, likely because scats from the rendezvous site were not analyzed. The under estimation of the number of individuals during the summer is likely due not only to the small sample size (few samples were good for the genetic analysis due to the degree of freshness and DNA degradation), but also due to the marking behavior of wolves. Because wolves are not leaving distinct trails as in the winter, it would be impossible to find scats off-trail. Scats collected in summer along human trails and roads were more likely samples from dominant or adult individuals that frequently mark the territory. These findings are consistent with wolf marking behavior (Peters and Mech 1975, Vila et al. 1994). However, it is not realistic to design a random sample for wolf scat collection during the summer to avoid this problem.

Recapture rates were variable: some genotypes were collected several times, whereas others were collected only once or twice. Genotypes sampled only once were from winter scats collected along wolf travel routes. Following snow tracks of a pack, it is possible to collect scats of each individual in spite of the individual marking behavior and non-random sampling. Therefore, every year individuals in the pack were better monitored during the winter.

Agreement between the 3 techniques is important to give more reliable results, and to evaluate errors of over/under estimation for each method. The techniques provided conflicting information during the first winter. I found evidence for the presence of 5 wolves from snow-tracking, while I identified 7 different individuals from genetic analysis. This lack of agreement in information may be due to difficulty in reading multiple tracks, and the possibility of movements of solitary wolves through the pack territory. Snow-tracking and genetic results agreed during the second winter, except for wolf M24, which was not genetically sampled, but was later found dead. The techniques provided similar results during the third winter. Wolf-howling estimations differ from the genetic results. Genetic results provided a low biased estimate during the summer, whereas during the winter genetic results agreed with the wolf-howling results of the previous summer.

Relatedness

Wolves in a pack are known to be partially related (generally except the alphas), sharing alleles that are identical by descent (Mech 1970, Lehman et al. 1992), and this corresponds to what I found in the Valle Pesio wolf pack. However, the genealogical relationships are preliminary and should be reassessed with 15 microsatellites loci for every individual (Queller et al. 1993). For example, if considering only the first 6 microsatellites, M24 and M23 were likely brothers, however, considering 12 microsatellites M24 did not appear to be related at all to the Valle Pesio wolf pack (Table 4). Field data from snow-tracking and wolf-howling surveys helped in identifying the likely parents. Low allelic diversity and high relatedness reduce the likelihood of identifying the pedigree relationships in packs because it is usually difficult to discriminate parent-offsprings from full sib dyads (Marshall et al. 1998). Increasing the number of loci is important for reliable kinship analysis. Moreover, the relatedness and kinship analyses are extremely sensitive to genotyping errors and high variance in any relatedness estimation does not allow detailed interpretations, therefore the main kinship determination should be based on confirmation from field observations.

Optimal sampling design

Genetic analysis on scats alone did not provide sufficient or adequate data to determine the wolf pack dynamics. The combination of data from snow-tracking surveys along with data from newly emerging DNA-based techniques provides a comprehensive view of the hidden life of wolves. The sampling design for scat collection should be evaluated based on the objectives of the study. If the principal goal is to follow the population dynamics of the packs, then the optimal sampling design should focus on intensive scat sampling along wolf travel routes during the winter season. This would allow for the collection of sufficient amounts of fresh scat, as well as related snow-tracking information. In the summer, if the scat collection at the rendezvous sites is not possible because of potential disturbance, it should be avoided. Wolf-howling is important to document reproduction, datum not detectable from genetic information. The approach described can be easily adapted to large areas with more packs, which would require more field personnel (e.g. forestry guards, park guards, volunteers). In this perspective, snow-tracking, and wolf-howling surveys, combined with genetic analysis of scat samples demonstrate the potential for a non-invasive wolf monitoring program over the Alps range. In the future genetic mark-recapture techniques might prove useful in estimation of population parameters (Mills et al. 2000, Waits and Leberg 2000, Pearse et al. 2001).

Wolf Pack Dynamics

When wolves appeared in the southwestern Alps far from known occupied wolf ranges in the Apennines Mountain, questions arose regarding the origin of the animals. Managers, hunters, and the public raised questions on the entity of the wolf population in the Alps. The genotypes identified in the Valle Pesio and Lugo wolf packs showed the exclusive presence of the Italian wolf haplotype W14 in all the sequenced DNAs (Randi et al. 2000). This suggests that the ongoing recolonization of the Alps in Italy and France is due to the natural expansion of the Italian wolf population in the Apennines Mountains.

The determination of population trends and an assessment of vital rates are necessary to understand the population dynamics of any species (Mech 1977). The most important parameters for population dynamics studies are reproduction, mortality, immigration, and dispersal rates (Gotelli 1998), data completely lacking in an Italian context (Boitani and Ciucci 1993).

Valle Pesio wolf pack size from 1999 to 2002 remained stable, with the presence of 5-6 individuals on average. In recolonizing wolf populations, pack size is expected to increase following pair formation and breeding (Fritts and Mech 1981), whereas the situation of the Valle Pesio pack seemed to be stable, likely due to a stabilized period after recolonization, which began almost 10 years ago. Despite the stable situation between years, more wolves were found at the beginning of the winter season (7 in 1999, 8 in 2000, and 6 in 2001) than at the end (5 in 1999, 6 in 2000, and 2-4 in 2001). This was likely due to an overlap at the beginning of the winter

(October-December) between yearlings and adults not yet dispersed, and pups (Fuller 1989).

Natural regulation of pack size through dispersal and mortality has been widely documented (Fuller 1989, Pletscher et al. 1997). Although there was a consistent pack size of 5-6 individuals during each year, there was a high yearly turn-over of individuals within the pack, never documented before in an Italian context. I could not distinguish between dispersal and mortality, but reproduction was documented each summer. By means of only snow-tracking, I could think that the same pack with the same individuals was present every year, whereas by means of only genetic analysis I could think that 15 different individuals were simultaneously present in the pack. Only by combining the 3 techniques I could reliably document a high turn-over of individuals.

There was a high apparent mortality rate in the pack, where I could not distinguish between dispersal and natural or human-caused mortality. While high dispersal rates for yearling wolves have often been reported (Fritts and Mech 1981, Fuller 1989, Gese and Mech 1991), especially in recolonizing populations (Boyd et al. 1992), pup dispersal is less common and generally limited to special circumstances such as high wolf density or high proportion of pups in the pack (Fuller 1989, Gese and Mech 1991). Therefore, pup disappearance was likely due to human-induced mortality or a disease able to reduce pup survival, such as for M23 and M24. Five wolves in the pack either dispersed or died throughout the study period. Although clear evidence of illegal mortality was not obtained, several anecdotes were reported of wolves systematically shot during wild boar drive hunts (November-January).

Frequent illegal mortality events during wild boar hunts have been reported elsewhere in Italy (Boitani and Ciucci 1993, Ciucci and Boitani 1999). I noticed solitary, exploratory forays of M2 in March 2000, before his disappearance. F20, during April 2002, had the same behaviour, moving 20 km away from the centre of the territory. Boyd and Pletscher (1999) evidenced that most wolves did not make exploratory forays 3 months before permanent separation from their natal pack, therefore M2 and F20 could be 2 potential dispersers.

Immigration into a population is difficult to monitor with certainty, even in intensively monitored populations (Pletscher et al. 1997). However, individuals genetically characterized, could be easily identified as immigrants. In winter 2002 wolf M25, an immigrant male, genetically different from the individuals in the Valle Pesio pack, joined the pack. The pack was composed of 6 females prior to the arrival of M25. The preponderance of females appeared to facilitate the immigration of a male into the pack.

Reproduction of the Valle Pesio wolf pack was documented every summer. M1 and F3 are the candidate parents for 1999, 2000, and 2001 pups from kinship analysis, exclusion analysis and field observation. M25 likely substituted for M1 in 2002, and was the father of the 2002 pups, while F3 likely maintained her dominant position. Ongoing genetic analysis of summer 2002 will elucidate this hypothesis. The Valle Pesio wolf pack never selected the same rendezvous site, even though the alpha animals were the same for the first 3 years.

Uncertainty concerning the relative roles of dispersal and mortality highlights the need to study wolves over a larger area (Ciucci and Boitani 1999) using radiotelemetry to document dispersal and mortality (Mech 1977). Moreover, the documentation of unrelated individuals in the pack for short periods, such as F21 and M24, shows the need for further investigation into the importance of issues such as short-term immigrations and movements of solitary wolves through the pack territory.

The role of each pack in the overall population could highlight the presence of population sources or sinks (*sensu* Pulliam 1988). Given the relevance of source-sink patterns for the conservation of wolves along the Alps, large-scale studies of pack dynamics based on methods which allow monitoring the social history of individual wolves are of paramount importance. However, few studies have measured dynamics of local populations and also considered patch size and interpatch distance at a large spatial scale (Hanski 1994). The concept of habitat sources and sinks for populations is popular, but rigorous tests are still uncommon.

Wolves apparently move throughout the landscape, across many unfavourable areas, but establishment success is restricted to higher quality habitats (Mladenoff et al. 1995). Regional landscape analysis and prediction of favourable wolf habitats have 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 studies emphasize the importance of long term monitoring data and large scale analysis to resolve complex spatial questions in resource management and conservation. The limitation of this approach is that presence-absence data were used to define favourable wolf habitat. It is not possible to infer habitat quality and population dynamics (Mladenoff et al. 1995 suggest a source-sink dynamic situation) because the models are not based on population parameters such as reproductive or survival rates.

Landscape models do allow practical conservation planning over large areas (Corsi et al. 1999). Future predictive mapping projects will be more useful if they are based on population parameters and not on just presence-absence data. Landscape models will be connected to mechanistic theories that predict population dynamics as a function of landscape attributes (Kareiva and Wennergren 1995). Given the difficulty in obtaining population parameter data over large scales, it would be appropriate to model wolf distribution over a large scale and then test population parameters within favourable and unfavourable habitats. This will allow inference about habitat quality and not only about habitat preference over a large scale. Information on landscape pattern is important for assessing the persistence of spatially structured populations. Endangered species, such as wolves that occupy fragmented habitats in Europe, may be tied to landscape pattern; therefore, conservation planning should be improved by this approach.

LITERATURE CITED

Boitani, L. 1976. Il lupo in Italia: numero, distribuzione e prime ricerche sulla sua ecologia ed etologia. Pages 7-42 in F. Pedrotti, editor. Sos Fauna, WWF, Camerino.

Boitani, L., and P. Ciucci. 1993. Wolves in Italy: critical issues for their conservation. Pages 74-90 in C. Promberger, and W. Schroder, editors. Wolves in Europe. Status and perspectives. Munich Wildlife Society, Ettal, Germany.

Boitani, L., and P. Ciucci. 1996. Stato delle conoscenze del lupo (*Canis lupus*) in Italia: prospettive di ricerca e conservazione. Pages 15-30 in F. Cecere, editor. "Dalla parte del lupo", WWF Italia.

Boyd, D., P. C. Paquet, S. Donelon, R. Ream, D. H. Pletscher, and C. C. White. 1992. Transboundary movements of a recolonizing wolf population in the Rocky Mountains. Pages 135-140. Ecology and conservation of wolves in a changing world, Edmonton.

Boyd, D., and D. H. Pletscher. 1999. Characteristics of dispersal in a colonizing wolf population in the Central Rocky Mountains. Journal of Wildlife Management **63**:1094-1108.

Carbyn, L. N. 1974. Wolf predation and behavioral interactions with elk and other ungulates in an area of high prey density. Page 233. Can. Wildl. Ser.

Ciucci, P. 1994. Movimenti, attività e risorse del lupo (Canis lupus) in due aree dell'Appennino centro-settentrionale. Page 117. University of Roma "La Sapienza", Roma, Italy.

Ciucci, P., and L. Boitani. 1999. Nine-year dynamics of a wolf pack in the Northern Apennines (Italy). Mammalia **63**:377-384.

Ciucci, P., L. Boitani, F. Francisci, and G. Andreoli. 1997. Home range, activity and movements of a wolf pack in central Italy. Journal of Zoology **243**:803-819.

Corsi, F., E. Duprè, and L. Boitani. 1999. A large-scale model of wolf distribution in Italy for conservation planning. Conservation Biology **13**:150-159.

Fritts, S. H., and L. D. Mech. 1981. Dynamics, movements, and feeding ecology of a newly protected wolf population in North-western Minnesota. Wildlife Monographs **80**:1-78.

Fuller, T. K. 1989. Population dynamics of wolves in North-Central Minnesota. Wildlife Monographs **105**:1-41.

Fuller, T. K., and B. A. Sampson. 1988. Evaluation of a simulated howling survey for wolves. Journal of Wildlife Management **52**:60-63.

Gagneux, P., C. Boesch, and D. S. Woodruff. 1997. Microsatellite scoring errors associated with non-invasive genotyping based on nuclear DNA amplified from shed hair. Molecular Ecology **6**:861-868.

Gese, E. M., and L. D. Mech. 1991. Dispersal of wolves (*Canis lupus*) in north-eastern Minnesota, 1969-1989. Can. J. Zool. **69**:2946-2955.

Goodnight, K. F., and D. C. Queller. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. Molecular Ecology **8**:1231-1234.

Gotelli, N. J. 1998. A primer of ecology. Sinauer Associates, Inc., Sunderland, Massachusetts.

Haight, R. G., and L. D. Mech. 1997. Computer simulation of vasectomy for wolf control. Journal of Wildlife Management **61**:1023-1031.

Hanski, I. 1994. A practical model of metapopulation dynamics. Journal of Animal Ecology **63**:151-162.

Harrington, F. H., and L. D. Mech. 1982. An analysis of howling response parameters useful for wolf pack censusing. Journal of Wildlife Management **46**:686-693.

Kareiva, P., and U. Wennergren. 1995. Connecting landscape patterns to ecosystem and population processes. Nature **373**:299-302.

Kohn, M. H., and R. K. Wayne. 1997. Facts from feces revisited. Tree **12**:223-227.

Kunkel, K. E. 1997. Predation by wolves and other large carnivores in northwestern Montana and southeastern British Columbia. Page 272. University of Montana, Missoula.

Lehman, N., P. Clarkson, L. D. Mech, T. J. Meier, and R. K. Wayne. 1992. A study of the genetic relationships within and among wolf packs using DNA fingerprinting and mithocondrial DNA. Behav. Ecol. Sociobiol. **30**:83-94.

Linnell, J. D. C., J. E. Swenson, A. Landa, and T. Kvam. 1998. Methods for monitoring European large carnivores. A worldwide review of relevant experience. Pages 1-38. NINA - Norsk institutt for naturforskning, Trondheim.

Litvaitis, J. A. 2000. Investigating food habits of terrestrial vertebrates in L. Boitani, and T. K. Fuller, editors. Research techniques in animal ecology. Controversies and consequences. Columbia University Press, New York.

Lucchini, V., E. Fabbri, F. Marucco, S. Ricci, L. Boitani, and E. Randi. 2002. Non-invasive molecular tracking of colonizing wolves (*Canis lupus*) packs in the Western Italian Alps. Molecular Ecology **11**:857-868.

Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology **7**:639-655.

Mattioli, L., M. Apollonio, V. Mazzarone, and E. Centofanti. 1995. Wolf food habits and wild ungulate availability in the Foreste Casentinesi National Park, Italy. Acta Theriologica **40**:387-402.

Mech, L. D. 1970. The wolf. The ecology and behavior of an endangered species. University of Minnesota Press, Minneapolis.

Mech, L. D. 1977. Productivity, mortality, and population trends of wolves in northeastern Minnesota. Journal of Mammology **58**:559-574.

Mills, L. S., J. J. Citta, K. P. Lair, M. K. Schwartz, and D. A. Tallmon. 2000. Estimating animal abundance using non-invasive DNA sampling: promise and pitfalls. Ecological Applications **10**:283-294.

Millspaugh, J. J., and J. M. Marzluff 2001. Radio Tracking and Animal Populations. Academic Press, San Diego.

Mladenoff, D. J., and T. A. Sickley. 1998. Assessing potential grey wolf restoration in the North-eastern United States: a spatial prediction of favourable habitat and potential population levels. Journal of Wildlife Management **62**:1-10.

Mladenoff, D. J., T. A. Sickley, R. G. Haight, and A. P. Wydeven. 1995. A regional landscape analysis and prediction of favourable gray wolf habitat in the Northern Great Lakes Region. Conservation Biology **9**:279-294.

Mladenoff, D. J., T. A. Sickley, and A. P. Wydeven. 1999. Predicting gray wolf landscape recolonization: logistic regression models vs. new field data. Ecological Applications **9**:37-44.

Pearse, D. E., C. M. Eckerman, F. J. Janzen, and J. C. Avise. 2001. A genetic analogue of 'Mark-recapture' methods for estimating population size: an approach based on molecular parentage assessments. Molecular Ecology **10**:2711-2718.

Peters, R., and L. D. Mech. 1975. Scent-marking in wolves. American Scientist **63**:628-637.

Pletscher, D. H., R. Ream, D. Boyd, M. W. Fairchild, and K. E. Kunkel. 1997. Population dynamics of a recolonizing wolf population. Journal of Wildlife Management **61**:459-465.

Pulliam, H. R. 1988. Sources, sinks, and population regulation. American Naturalist **132**:652-661.

Queller, D. C., J. E. Strassmann, and C. R. Hughes. 1993. Microsatellites and Kinship. Trends in Ecology and Evolution **8**:285-288.

Randi, E., V. Lucchini, M. F. Christensen, N. Mucci, S. M. Funk, G. Dolf, and V. Loeschckes. 2000. Mitochondrial DNA variability in Italian and East European wolf: detecting the consequences of small population size and hybridazion. Conservation Biology **14**:1-11.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L. P. Waits, and J. Bouvet. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research **26**:3189-3194.

Taberlet, P., L. P. Waits, and G. Luikart. 1999. Non-invasive genetic sampling: look before you leap. TREE **14**:323-327.

Tucker, P., D. L. Davis, and R. Ream 1990. Wolves. Identification, documentation, population monitoring, and conservation considerations. Northern Rockies Natural Resource Center of the National Wildlife Federation, Missoula.

Vila, C., V. Urios, and J. Castroviejo. 1994. Use of faeces for scent marking in Iberian wolves (Canis lupus). Canadian Journal of Zoology **72**:374-377.

Waits, L. P., and P. L. Leberg. 2000. Biases associated with population estimation using molecular tagging. Animal Conservation **3**:191-199.

White, K. A. J., M. A. Lewis, and J. D. Murray. 1996. A model for wolf-pack territory formation and maintenance. J. Theor. Biol. **178**:29-43.

Woods, J. G., D. Paetkau, D. Lewis, B. N. McLellan, M. Proctor, and C. Strobeck. 1999. Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin **27**:616-627.

Chapter 3. Winter Diet Selection of Wolves in the Western Alps: Optimization of a Sampling Design

Abstract: I estimated the diet of the Valle Pesio wolf (*Canis lupus*) pack for 3-winter seasons (1999-2002) and evaluated winter wolf diet selection along wolf travel routes. Roe deer (*Capreolus capreolus*) was the main prey species for wolves in the area. Diet components differed between winters ($\underline{P}<0.001$); relative use of red deer (*Cervus elaphus*) was greater in winter 2001, while use of wild boar (*Sus scrofa*) was greater in winter 2002 than during other winters. I evaluated possible biases in the scat collection method and determined an optimal sampling design using Monte Carlo simulations. Clusters of scat from the same kill site did not bias the total sample; however, scats that were not independent could introduce errors in the diet estimation. Therefore, the best sampling design to reduce a possible bias was the "additive method," where a collection of scats along a wolf travel route was considered one sampling unit to avoid pseudo-replication. The final additive sample was more representative because it included kills and scat collection data. I examined changes in resolving power with respect to effort expended for a range of possible sample sizes. The standard error was used as a measure of resolving power. Within the range, trade-offs between resolving power and expended effort were used for making the final choice of sample size. The procedure provided a tool for optimizing sampling design of scat collection.

INTRODUCTION

Recent studies have examined the diet of wolves in Europe (Smietana and Klimek 1993, Ciucci 1994, Papageorgiou et al. 1994, Mattioli et al. 1995, Poulle et al. 1995a, Jedrzejewski et al. 2000) and North America (Potvin et al. 1988, Huggard

1993b, Marquard-Petersen 1998) using scat analyses methods. However, few researchers examined the reliability and the limitations of their techniques.

Scat content analysis to assess carnivore diets is widely used because the approach is inexpensive, easy to apply, and large samples can be collected (Litvaitis 2000). The method is non-intrusive, non-destructive, and thus compatible with the endangered status of the wolf in most countries (Ciucci et al. 1996). However, scat analysis has a limited scope of inference: it is not possible to differentiate between scavenged or killed prey (Ciucci 1994) and it is difficult to record resource use at the individual level (Manly et al. 1993).

Diet analysis presents both technical and analytical difficulties (Reynolds and Aebischer 1991) and biases can occur at each step of the process (Litvaitis et al. 1996). Diet methods are organized into 3 main steps: scat collection, scat analysis, and data analysis. Although biases and interpretational difficulties have been considered for scat analysis and data analysis (Reynolds and Aebischer 1991, Ciucci et al. 1996), scat collection has rarely been discussed. Estimates may be biased because of non-random sampling (Manly 1996). Random sampling is often difficult to implement, and a temptation exists to assume that a sample that is obtained in some convenient way is equivalent to a random sample (Manly 1996). As a result, systematic bias in the sampling procedure may distort estimates of key parameters (Manly 1996).

Potential biases associated with scat collection include the design of field sampling, non-wolf scat inclusion in the analysis, independence of the data, and the definition of a sampling unit (Carss and Parkinson 1996).

Random sampling of wolf scats in the field is not feasible; therefore opportunistic scat sampling along human trails and roads is usually conducted (e.g.

Ciucci 1994, Papageorgiou et al. 1994, Mattioli et al. 1995, Poulle et al. 1995a). Potential biases may occur if opportunistic scat sampling produces an unrepresentative sample or if sampling scats at rendezvous or kill sites causes overrepresentation of a prey species in the diet.

In most field conditions, no single criterion allows distinction between scats of wolves and other canids (Litvaitis et al. 1996). A conservative, multi-criteria approach to differentiate wolf scats from those of other canids is often used (Ciucci 1994, Poulle et al. 1995a). However, possible biases due to including non-wolf scat or discarding some wolf scat may occur.

A basic assumption when determining diet by frequency of occurrence is that each occurrence of a prey species in different scats represents an independent sample (Carss and Parkinson 1996). Scats collected along a wolf travel route or at a kill site often are not independent (Hurlbert 1984, Huggard 1993b).

A sample of scats that is small compared with the total number of scats produced may be unrepresentative (Reynolds and Aebischer 1991). The resulting standard error in assessing any aspect of the diet will depend on the variability between scats and on the size of the sample (Reynolds and Aebischer 1991). An essential component of any experimental or sampling design is the choice of an appropriate sample size (Bros and Cowell 1987). If the sample size is too small, the power of the test is likely to be insufficient for hypothesis testing (Bros and Cowell 1987). Conversely, when the sample size is too large the power of a specific test may be adequate, but effort may be wasted in collecting and processing samples.

I estimated the diet of the Valle Pesio wolf pack by analyzing scats and by locating kills during 3-winter seasons in the southwestern Alps. I estimated prey use by wolf diet data and prey availability by ungulate track counts along wolf travel routes (Kunkel 1997). I evaluated wolf diet selection by comparing use and availability (Manly 1974, Chesson 1978, Thomas and Taylor 1990, Krebs 1998). I evaluated the possible biases that can occur in each part of the methods and interpreted the limitations and elucidated the nature of the biases involved. I proposed a method in which the range of sample sizes is limited at the low end by an acceptable level of resolving power, and at the high end by feasibility (Bros and Cowell 1987). Within this range, trade-offs between resolving power and expended effort are used for making the final choice of sample size (Bros and Cowell 1987).

Therefore my main objectives were to:

1- Estimate wolf diet selection along wolf travel routes.

2- Design an optimal sampling method for scat collection.

A good sampling design with the best cost-benefit approach is fundamental and interpretation among and comparability between diet studies would benefit from a better understanding of the sources of error associated with each step of the analysis.

STUDY AREA

The study area was defined by the Valle Pesio wolf pack territory and is located in a mountainous region of the southwestern Alps of Italy and France. The area is approximately 500 km^2 and encompasses the Alta Valle Pesio e Tanaro Natural Park (67.7 km^2) and adjacent lands. The core area is characterized by long narrow valley bottoms surrounded by rugged mountains with elevations ranging from 800-2651 m. Coniferous and broadleaf forests (*Abies alba, Fagus sylvatica* are prevalent) cover about 50% of the area; 30% consists of alpine meadows, and 20% of shrubs and rocky areas. The few roads in the area are closed during winter. Annual average precipitation is 1285 mm and the snow-season generally goes from

November to April. Human presence is low in the area due to a steady and constant decline in the population during the past 30 years. However, a high human density exists in the region. The study area encompassed parts of Italy and France, and each country consists of a mosaic of land management classes. The most common ungulate species in the area are chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), and red deer (*Cervus elaphus*). Populations of roe deer, chamois, and wild boar in the study area today are abundant as a consequence of reintroductions by the Italian and French Park Systems beginning in the 1980s and of natural range expansion by ungulates throughout Italy (Mattioli et al. 1995). Smaller mammalian prey species include marmot (*Marmota marmota*), marten (*Martes martes*), and hare (*Lepus europeus*).

METHODS

Wolf diet selection

I determined wolf diet by analyzing scats and by locating kills during 3 winter seasons (1999-2002). I estimated prey use through wolf diet analysis and prey availability using systematic transects along wolf travel routes. I evaluated wolf diet selection by comparing use and availability (Manly 1974, Chesson 1978, Thomas and Taylor 1990, Krebs 1998).

Scat collection and analysis

I backtracked wolves during 3 winters (1999-2002) to collect scats. On days following snowfall, I searched for wolf tracks in the area on skis or snowshoes. When I found wolf tracks, I followed their travel routes first in the opposite direction of wolf travel and then on subsequent days I continued following wolf routes in 1) the same direction, and 2) in the direction of wolf travel, avoiding disturbing the wolves

(Kunkel 1997). In this way, I travelled a wolf travel route on different days and I considered each continuous route a "snow-tracking session" (Ciucci 1994). I collected each wolf scat that I encountered, and froze it (-30°C) prior to analysis. Scats were also occasionally collected during other winter field activities. I only collected scats of the Valle Pesio wolf pack.

I followed the laboratory procedures of Reynolds and Aebischer (1991) (Appendix A). I identified mammalian hairs in each scat by microscopic examination of the cuticular pattern, the medulla, and the cross-section. I assessed accuracy and consistency of observers in identifying mammalian hairs (Fritts and Mech 1981; Appendix A).

I used the following techniques to assess the diet of wolves:

1- Frequency of occurrence (Scott and Schackleton 1980, Ballard et al. 1987). I expressed the frequency with which each food item occurred as a percentage of the total number of occurrences of all food items, rather than as a percentage of the total number of scats. The former measure is more meaningful in terms of diet composition because it expresses the frequency of a food item relative to the other food items recovered in the scat sample (Ciucci et al. 1996).

2- Biomass ingested, using a model obtained through feeding trials with packs of captive Italian wolves and prey (Ciucci et al. 2001). I estimated the biomass ingested using a linear model of the known relationship between prey biomass consumed per collectable scat produced:

$y = 0.274 + 0.011x$ ($R^2=0.74$, $\underline{P}<0.001$)

where x = the live weight of the prey species identified in the scat

y = kg of biomass ingested/collectable scat

To account for scats containing more than one prey item, I visually estimated the relative proportions of an individual prey species in the scat to the nearest 1% and summed the proportions for all scats where that prey species was recovered (Ciucci 1994). I estimated live weights of the prey species from the literature (Perco 1986).

I generated 95% non-simultaneous bootstrap confidence limits for the percentage of each food item in the diet. These limits represent the effects of random sampling error (Manly 1998). I simulated 2000 sets for each bootstrap simulation.

I evaluated differences in the diet between each winter scat-sample using a chi-square test on frequency of occurrence data (Reynolds and Aebischer 1991). Data were pooled for years when the diets were similar.

Wolf kills

I backtracked wolves during 3 winters (1999-2002) to locate prey carcasses (Huggard 1993a, Kunkel et al. 1999). Additional ungulate carcass and scavenging information were obtained from Park personnel. Evidence for a wolf kill included an obvious chase sequence in the snow or evidence of a struggle, including blood on the snow, and/or subcutaneous haemorrhaging at wound sites (Mech 1970, Huggard 1993c). Only confirmed wolf kills were included in the analysis. I examined each carcass for cause of death, species, age, and sex of prey killed, and degree of consumption (Boyd et al. 1994).

Prey availability along wolf travel routes

I used systematic transects to estimate relative proportions of prey along wolf travel routes (Kunkel 1997). I skied 2 100 m transects in opposite directions and perpendicular to the wolf travel route at 1 km intervals. The location of the first transect was chosen randomly. The distance to the first roe deer, chamois, red deer, or wild boar on each transect was recorded; if no track was encountered the distance recorded was 100 m. The number of prey tracks located on both transects (0, 1 or 2; only the first track on each transect was recorded) was divided by the distance to that track (e.g. 1/190 if one roe deer track is found at 90 m in one direction and if no tracks are found in the opposite direction) to obtain the number of prey tracks/m. This value was divided by the number of days since the most recent snowfall > 5 cm to adjust for snowfall effects (Kunkel 1997). I did not ski transects after more then 7 days had elapsed since a snowfall because track deposition plateaued and tracks deteriorated after this time (Kunkel 1997).

Wolf diet selection

I calculated Manly's (1974) index α for each prey species by using the constant prey population method (Krebs 1998) to estimate the dietary preference of the Valle Pesio wolf pack:

Manly's index: $\alpha_i = (r_i / n_i)\ [1 / \sum_{i=1}^{m} (r_i / n_i)]$;

where r_i = proportion of prey i in the diet (i = 1,2,...,m)

n_i = proportion of prey i in the environment (i = 1,2,...,m)

m = number of prey species possible

I normalized α values so that their sum equalled 1.0. Thus, if predation is not selective, $\alpha = 1/m$; if a prey item is preferred, $\alpha > 1/m$ (Manly 1974).

Sampling design for scat collection

I assessed the accuracy of the scat collection method in relation to different sources of bias (design of field sampling, non-wolf scat issues, and statistical independence of the data) and precision (sample size).

Bias

I compared the wolf diet based on scats collected only along human trails to the wolf diet based on scats collected along wolf travel routes using chi square test using frequency of occurrence data. I assumed that the sampling design on wolf travel routes was more accurate and representative than the sampling design based only on human trails. I also evaluated if the inclusion of data from scats found at kill-sites caused over-representation of a prey species.

I used a conservative, multi-criteria approach to differentiate wolf scats and tracks from those of other canids (Ciucci 1994). However, possible biases due to including non-wolf scats in the samples may still have occurred. Therefore, I sent a sample of the uncertain wolf scat sample to the Instituto Nazionale della Fauna Selvatica (INFS) genetic laboratories in Bologna (Italy) for genetic analysis to discriminate between different canid species (Randi et al. 2000). I evaluated the proportion of non-wolf scats present in the sample collection.

Scats collected in the same scat collection often contain hair from the same kill and are not independent samples (Huggard 1993b). Wolves within a pack eat together and deposit scats along the same travel route. Therefore, I pooled wolf scats into "collections" defined as the aggregate of wolf scats collected along a continuous wolf travel route, uninterrupted by an identifiable carcass, or by lack of snow. Therefore, a collection of scats, and not a single scat, was considered a sampling unit for the diet analysis to avoid pseudo-replication (Hurlbert 1984).

Carcasses of prey found represented a known minimum amount of prey consumed for the wolf pack. I supplemented the list of wolf carcasses found during snow-tracking sessions with the data from scat collections. I estimated the intervals between discovered carcasses by scat collection analysis. I assumed a missed carcass when scat collection from a certain day contained hair of a species different from the known preceding kill, and/or > 3 days from the preceding known kill of that species (Huggard 1993b, Jedrzejewski et al. 2002). In a controlled feeding experiment, Floyd et al. (1978) found that wolves defecated undigested prey remains 8-56 h (0.3-2.3 days) after consumption. I estimated the date of defecation for each scat based on wolf snow-tracking data and scat freshness (Jedrzejewski et al. 2002). In this way, I reduced serial correlation on the contents and made the scats and carcass data additive. This "additive method" should have less bias and address the independence issue. I evaluated the diet selection of the Valle Pesio pack using the additive data for prey use estimation. I calculated Manly's (1974) index of selection α for each prey species by using the constant prey population method (Krebs 1998). I estimated standard errors of the α values following Manly (1974).

Precision

I estimated the true scat population of the Valle Pesio wolf pack for the 3 winter seasons (1999-2002), based on the number of wolves present in the pack, a defecation rate of 2 scats/wolf/day, and a winter season of 181 days (November-April). I excluded from the analysis scats belonging to individuals of other packs. I used a defecation rate of 2 scats/wolf/day, based on a defecation rate of 4 scats/wolf/day for captive wolves fed *ad libitum* for short periods (Floyd et al. 1978, Weaver 1993) and considering that wild wolves eat variably, likely reducing the mean number of scats produced per day (Tosoni 2002).

I measured the precision of dietary estimates through an analysis of the sampling variance, which is measured by the squared standard error. I determined the sample size necessary for a chosen level of precision using a form of Monte Carlo simulation (Reynolds and Aebischer 1991:114). I used the scat samples of each winter to provide variance estimates to determine the standard error curve (Bros and Cowell 1987).

The Monte Carlo procedure I used to generate the standard error function was a sampling procedure from a realistic, finite population, generated using resampling without replacement (Snedecor and Cochran 1980). For each sample size, I randomly drew 1000 samples from the estimated scat population and computed the standard error (Appendix B). This number of resamples was determined to be a realistic minimum for a test of significance at $\alpha = 0.05$ (Manly 1998).

I evaluated the sampling effort to look for wolf tracks and to follow tracks for collecting scats for each winter season. I considered the effort as days of work for one person. I examined changes in power with respect to effort expended for a range of possible sample sizes bounded by the minimum acceptable standard error at the low end and feasibility at the high end. Within the range, I used trade-offs between resolving power and expended effort for making the final choice of sample size (Bros and Cowell 1987).

RESULTS

Wolf Diet Selection

Prey use

I collected 435 scats along 694.1 km of wolf tracks (112 scats during winter 1999-2000 [winter 00], 179 during winter 2000-2001 [winter 01], and 144 during winter 2001-2002 [winter 02]).

The winter diet of the Valle Pesio pack differed significantly by year (frequency of occurrence: $\chi^2=74.01$, df=8, $P<0.0001$; biomass: $\chi^2=76.13$, df=8, $P<0.0001$). Therefore, I did not pool the diet data for years.

Wild ungulates (especially roe deer) composed the majority of the diet during each winter (85.5%, 89.1%, 98.1% of the total biomass consumed; Table 1).

Domestic mammals (goats, sheep, and dogs) were of limited importance as were small mammals (Table 1).

Table 1. Diet composition and food items ranked by 2 criteria for the Valle Pesio wolf pack during 3 winter seasons (1999-2002), in the southwestern Alps of Italy and France.

	Frequency of occurrence			Biomass (Ciucci et al. 2001)		
WINTER 00						
	n	%	rank	kg	%	rank
Roe deer	60	48.8	1	29.5	45.7	1
Chamois	29	23.6	2	14.9	23.1	2
Goat/Sheep	12	9.8	3	6.2	9.6	4
Wild boar	11	8.9	4	8.0	12.4	3
Dog	7	5.7	5	3.0	4.6	5
Red deer	2	1.6	6.5	2.7	4.3	6
Small mammals	2	1.6	6.5	0.2	0.3	7
Total	123	100		64.6	100	
WINTER 01						
	n	%	rank	kg	%	rank
Roe deer	106	54.6	1	55.5	44.6	1
Red deer	26	13.4	2	31.4	25.2	2
Chamois	22	11.4	3.5	13.7	11.0	3
Goat/Sheep	22	11.4	3.5	12.8	10.3	4
Wild boar	15	7.7	5	10.4	8.3	5
Dog	2	1.0	6	0.8	0.6	6
Small mammals	1	0.5	7	0.0	0	7
Total	194	100		124.6	100	
WINTER 02						
	n	%	rank	kg	%	rank
Roe deer	72	47.7	1	34.2	35.7	2
Wild boar	45	29.8	2	43.4	45.2	1
Chamois	28	18.5	3	15.0	15.6	3
Goat/Sheep	3	2.0	4	1.3	1.4	5
Red deer	2	1.3	5	1.6	1.7	4
Dog	1	0.7	6	0.4	0.5	6
Total	151	100		64.6	100	

Roe deer occurrence in wolf scats was higher than the other prey species during each winter (Table 1). Within species, I found that red deer was more important in the winter 01 wolf diet, whereas wild boar was more important during winter 02.

The 2 methods of estimating wolf diets from scats provided very similar rankings of prey items in the diet for each winter season (Spearman's correlation: $0.929<r<0.991$; $0.0001<P<0.003$).

The non-simultaneous 95% bootstrap confidence intervals (Table 2) indicated a small sampling error for roe deer and chamois. The lower bound of the confidence intervals included zero for red deer in winters 00 and 02, small mammals and dogs in winters 01 and 02, and goats/sheep in winter 02.

Table 2. Results of bootstrap simulation (2000 repetitions) to generate 95% non-simultaneous confidence intervals for the proportion of each food item in a quantitative analysis of wolf diet, based on n=435 scats.

	Winter 00			Winter 01			Winter 02		
		Bootstrap95%CI			Bootstrap95%CI			Bootstrap95%CI	
	Mean	Lower	Upper	Mean	Lower	Upper	Mean	Lower	Upper
Roe deer	0.577	0.481	0.673	0.599	0.525	0.667	0.508	0.422	0.591
Chamois	0.278	0.192	0.365	0.124	0.079	0.175	0.196	0.127	0.261
Red deer	0.019	0	0.048	0.147	0.096	0.203	0.014	0	0.035
Wild boar	0.106	0.048	0.164	0.085	0.046	0.131	0.317	0.239	0.387
Dog	0.066	0.019	0.115	0.011	0	0.028	0.007	0	0.021
Goat/Sheep	0.116	0.058	0.183	0.125	0.079	0.176	0.021	0	0.049
Smallmammals	0.019	0	0.048	0.006	0	0.017			

I found 51 wolf kills (Winter 00: n=11; Winter 01: n=22; Winter 02: n=18) and 4 scavenging events along 694.1 km wolf tracks. Roe deer comprised the largest proportion of prey carcasses during every winter (Winter 00: 0.91; Winter 01: 0.69; Winter 02: 0.83) (Table 3). Although I found 51 wolf kills over 3 winters, the data on age structure and physical condition were limited. Many carcasses were incomplete because of consumption by wolves: the legs and the head were often missing.

Table 3. Prey carcasses of the Valle Pesio wolf pack during 3 winter seasons (1999-2002) in the southwestern Alps of Italy and France.

	Winter 2000		Winter 2001		Winter 2002	
	n	Proportion	n	Proportion	n	Proportion
Roe deer	10	0.909	18	0.692	15	0.833
Chamois	0	0	1	0.038	2	0.111
Red deer	0	0	5	0.192	0	0
Wild boar	1	0.091	2	0.078	1	0.056
Total	11	1	26	1	18	1

Prey availability

Roe deer tracks were more frequent than other ungulates tracks encountered on systematic transects along wolf travel routes (Table 4), for both winter 01 and 02.

Table 4. Prey species tracks along wolf travel routes as a measure of encounter availability for the Valle Pesio wolf pack, southwestern Alps of Italy and France, winters 2001-2002.

	Winter 2001 n = 85 transects		Winter 2002 n = 53 transects	
Species	Proportion	Tracks/km	Proportion	Tracks/km
Roe deer	0.550	9.10	0.445	5.95
Chamois	0.216	3.58	0.262	3.50
Wild boar	0.167	2.76	0.205	2.75
Red deer	0.067	1.10	0.089	1.18

Prey selection

Because 4 primary prey species occurred in my study area, α values of 0.25 indicated that use reflected availability. In Winter 01, wolves selected red deer (Manly's $\alpha = 0.678$) over roe deer (Manly's $\alpha = 0.147$), chamois (Manly's $\alpha = 0.094$), and wild boar (Manly's $\alpha = 0.081$) when use evaluated with biomass analysis of scats was compared to availability along systematic transects on wolf travel routes. If use was evaluated with the number of kills, wolves selected red deer (Manly's $\alpha = 0.602$) and roe deer (Manly's $\alpha = 0.264$) over wild boar (Manly's $\alpha = 0.097$), and chamois (Manly's $\alpha = 0.037$) (Table 5).

In Winter 02, wolves selected wild boar (Manly's $\alpha = 0.777$) over roe deer (Manly's $\alpha = 0.122$), red deer (Manly's $\alpha = 0.012$), and chamois (Manly's $\alpha = 0.091$) when use evaluated with biomass analysis of scats was compared to availability along systematic transects on wolf travel routes. If use was evaluated with the number of kills, wolves selected roe deer (Manly's $\alpha = 0.641$) over wild boar (Manly's $\alpha = 0.214$), and chamois (Manly's $\alpha = 0.145$) (Table 5).

Table 5. Proportions of prey available and prey used by wolves of Valle Pesio pack, during winter 2001 and winter 2002, southwestern Alps of Italy and France.

	Winter 2001			Winter 2002		
	Prey available	Prey use		Prey available	Prey use	
Proportion:	Tracks/km	Scats	Kills	Tracks/km	Scats	Kills
Roe deer	0.550	0.504	0.692	0.445	0.363	0.833
Chamois	0.216	0.127	0.038	0.262	0.159	0.111
Red deer	0.067	0.284	0.192	0.205	0.017	0
Wild boar	0.167	0.085	0.078	0.089	0.461	0.056

Sampling Design For Scat Collection

Bias

Wolf diet evaluated with scats found along hiking trails was similar to the wolf diet evaluated with the entire sample for each year (Winter 00: $\chi^2=0.416$, df=6, $\underline{P}=0.998$; Winter 01: $\chi^2=2.117$, df=6, $\underline{P}=0.909$; Winter 02: $\chi^2=0.485$, df=5, $\underline{P}=0.922$).

The inclusion in the overall analysis of data from scats found at kill-sites did not cause overrepresentation of the species found at those kill-sites in the diet. Approximately 67% of the kill sites did not have scats nearby. Average number (± 1 SD) of scats at a kill site was 0.6 ± 0.9 (range 0-3). Of those kill sites with scats, 50% of the scats contained hair from a species other than the kill.

Only 1 of the 150 scats genetically tested did not belong to a wolf. Inclusion of non-wolf scats occurred too infrequently to bias the diet analysis.

Scats found along a single travel route could belong to the same individual. In winter 01-02, 2 consecutive scats along the same travel route were from wolf F3 (both scats contained chamois hairs); and 3 consecutive scats were from wolf M25 (all scats contained roe deer hairs). These scats were not independent samples.

Mean number of scats per collection (± 1 SD) was 3.5 ± 2.5 (n=46) (range 1-10) (Winter 00: x ± 1 SD = 2.6 ± 1.3, n =16, range = 1- 6; Winter 01: x ± 1 SD = 4.3 ± 2.9, n =13, range = 1- 10; Winter 02: x ± 1 SD = 3.8 ± 2.7, n=17, range = 1- 9) (Table 6).

The analysis of additive data from scat collection and carcasses found along wolf travel routes from known dates indicated a minimum of 16 missed prey in Winter 00 (7 Roe deer, 5 chamois, 1 Red deer, and 3 wild boar), 13 missed prey in Winter 01 (6 Roe deer, 5 chamois, and 2 wild boar), and 16 missed prey in Winter 02 (4 Roe deer, 5 chamois, 1 Red deer, and 6 wild boar) (Table 6-7).

Winter 00			Winter 01			Winter 02		
ADDITIVE METHOD	**PREY SPECIES**	**DATE**	**ADDITIVE METHOD**	**PREY SPECIES**	**DATE**	**ADDITIVE METHOD**	**PREY SPECIES**	**DATE**
WK1	Roe deer	24-nov	WK15	Roe deer	19-nov	Scat collection (670-671)	Chamoix	13-nov
WK2	Roe deer	26-nov	Scat collection (383-391)	Goat	22-nov	WK46	Roe deer	13-nov
WK3	Roe deer	29-nov	Scat collection (392-396)	Wild boar	23-nov	WK47	Roe deer	18-nov
Scat collection (134-136)	Chamoix	04-dec	WK17	Chamoix	27-nov	Scat collection (685-687)	Chamoix	20-nov
Scat collection (141-144)	Roe deer	06-dec	WK18-19	2Wild boar	05-dec	WK48	Roe deer	20-nov
WK4	Roe deer	08-dec	Scat collection (399)	Roe deer	08-jan	WK49	Roe deer	02-dec
Scat collection (153-155)	Chamoix/Red deer	19-dec	Scat collection (401-407)	Chamoix	10-jan	Scat collection (698-699)	Chamoix	04-dec
WK5	Roe deer	26-dec	WK20-21	2Roe deer	10-jan	Scat collection (714)	Roe deer	16-dec
WK6	Wild boar	27-dec	WK22	Roe deer	12-jan	WK50	Roe deer	18-dec
Scat collection (163-164)	Chamoix	30-dec	WK23	Roe deer	17-jan	Scat collection (728-732)	Roe deer	27-dec
Scat collection (167-169)	Roe deer	08-jan	WK24	Roe deer	18-jan	WK51	Roe deer	28-dec
WK8	Roe deer	11-jan	Scat collection (427-432)	Goat	28-jan	WK52	Roe deer	02-jan
Scat collection (171-173)	Chamoix/Wild boar	12-jan	WK25	Red deer	28-jan	Scat collection (739)	Goat	06-jan
Scat collection (181-182)	Roe deer	17-jan	WK26	Roe deer	28-jan	Scat collection (740-747)	Wild boar	08-jan
Scat collection (184-189)	Roe deer	23-jan	WK27	Red deer	29-jan	WK53	Roe deer	10-jan
WK9	Roe deer	28-jan	WK28	Roe deer	30-jan	WK54	Wild boar	13-jan
Scat collection (200-201)	Wild boar	01-feb	WK29	Roe deer	31-jan	WK57	Roe deer	16-jan
Scat collection (203)	Goat	01-feb	WK30	Roe deer	04-feb	WK55	Roe deer	18-jan
Scat collection (206-207)	Goat	11-feb	WK31	Roe deer	06-feb	WK56	Chamoix	20-jan
Scat collection (216-219)	Roe deer	16-feb	Scat collection (452-453)	Chamoix	09-feb	Scat collection (768-769)	Roe deer	29-jan
WK11	Roe deer	28-feb	WK33	Roe deer	09-feb	Scat collection (773-774)	Wild boar	04-feb
WK12	Roe deer	10-mar	Scat collection (461-470)	Roe deer/Wild boar	19-feb	WK58-59	Roe deer	05-feb
Scat collection (234)	Chamoix	12-mar	Scat collection (475-478)	Roe deer	23-feb	WK60	Roe deer	10-feb
Scat collection (255-256)	Roe deer	28-mar	Scat collection (479-480)	Roe deer	04-mar	Scat collection (779-786)	Roe deer	22-feb
WK13	Roe deer	05-apr	WK38	Roe deer	06-mar	Scat collection (799-806)	Wild boar	09-mar
Scat collection (257)	Wild boar	13-apr	SC39	Roe deer	06-mar	WK61	Chamoix	12-mar
Scat collection (258-259)	Roe deer	19-apr	WK40	Roe deer	11-mar	WK62	Roe deer	12-mar
			SC41-42-43	3 Red deer	11-mar	WK63	Roe deer	13-mar
			WK44	Roe deer	19-mar	Scat collection (808-809)	Chamoix	19-mar
			Scat collection (527)	Chamoix	22-mar	Scat collection (806-808)	Wild boar	19-mar
			WK45	Roe deer	30-mar	814-816+818	Wild boar	03-apr
			Scat collection (545-548)	Roe deer	09-apr	Scat collection (821-829)	Wild boar	15-apr
			Scat collection (550-551)	Chamoix	15-apr	Scat collection (834-835)	Red deer	24-apr
			WK46	Roe deer	15-apr	Scat collection (844-846)	Chamoix	25-apr
			Scat collection (561-563)	Chamoix	26-apr	Scat collection (838-840)	Roe deer	30-apr

Table 6. Scat collection (number of scats), wolf kill (WK), and scavenging (SC) data addition on a chronological basis, to evaluate wolf food habits, during winter 00-01-02, of the Valle Pesio wolf pack in the southwestern Alps.

When prey use evaluated with additive data was compared to prey availability along systematic transects on wolf travel routes, in winter 01 wolves of the Valle Pesio pack selected red deer over chamois and wild boar, while roe deer were eaten as available (Table 7). In winter 02, wolves selected wild boar over chamois and red deer, while roe deer were eaten as available (Table 7).

Table 7. Prey eaten by wolves (evaluated by the additive method) and prey availability proportions along wolf travel routes used to determine wolf prey selection of the Valle Pesio pack using Manly's α values and standard error (SE), southwestern Alps, Italy and France.

	Winter 2001				Winter 2002			
	Eaten	Available	α	SE	Eaten	Available	α	SE
Chamois	6	0.216	0.165	0.067	7	0.262	0.175	0.065
Wild boar	4	0.167	0.142	0.068	7	0.089	0.514	0.107
Roe deer	23	0.550	0.249	0.067	19	0.445	0.279	0.074
Red deer	5	0.067	0.444	0.121	1	0.205	0.032	0.032

Precision

I estimated the minimum number of individuals per month of the Valle Pesio wolf pack through snow-tracking and genetic results for each winter season (Table 8).

Table 8. Estimate of the number of wolves in the Valle Pesio pack, per month, during 3 winter seasons (1999-2002).

	Winter 00	Winter 01	Winter 02
Month	N° individuals	N° individuals	N° individuals
November	5	8	5
December	5	6	5
January	5	6	5
February	5	6	2
March	5	5	2
April	a	4	2

a* Not enough snow to detect the number of wolves in the area

I estimated a total population of scats of 1510 for winter 00, of 1790 for winter 01, and of 1186 scats for winter 02. I used variance estimates of the diet from a sample of 112 scats in winter 00, 179 scats in winter 01, and 144 scats in winter 02 to determine the standard error curve. These scat samples represented 7.4%, 10%, and 12.1% of the respectively estimated real scat population.

A larger sample size of scats reduced the standard error and increased the precision of estimating frequency of occurrence in the wolf diet for each wild ungulate prey species (chamois, roe deer, red deer, and wild boar) during each winter (Appendix C). If I considered sample sizes of scats < 200, the 95% confidence interval for red deer included 0 in winters 00 and 02 (Appendix C). The standard error for each prey species declined rapidly until an n of approximately 200 scats was reached (Figure 2-3-4). Therefore, I should collect at least a sample size greater than 10-13% of the scat population to detect prey species that have a low percent occurrence in the diet, as did red deer in winter 00 and 02.

Sample size versus sampling effort

I evaluated a sampling effort of 191 days of work in winter 00 to collect 145 scats along wolf travel routes; of 162 days of work in winter 01 to collect 190 scats; of 262 days of work in winter 02 to collect 170 scats (Figure 1).

I need at least an effort of 250 days of work to collect around 200 scats. Therefore, I need 2 people to sample daily throughout each winter. In this way, the minimum acceptable sample size is beyond the region of greatest change in slope of the standard error function (Figure 2-3-4) (Appendix C).

Figure 1. Sampling effort (days of work by one person to collect scats along wolf travel routes) for each winter season (winters 2000-2002), southwestern Alps of Italy and France.

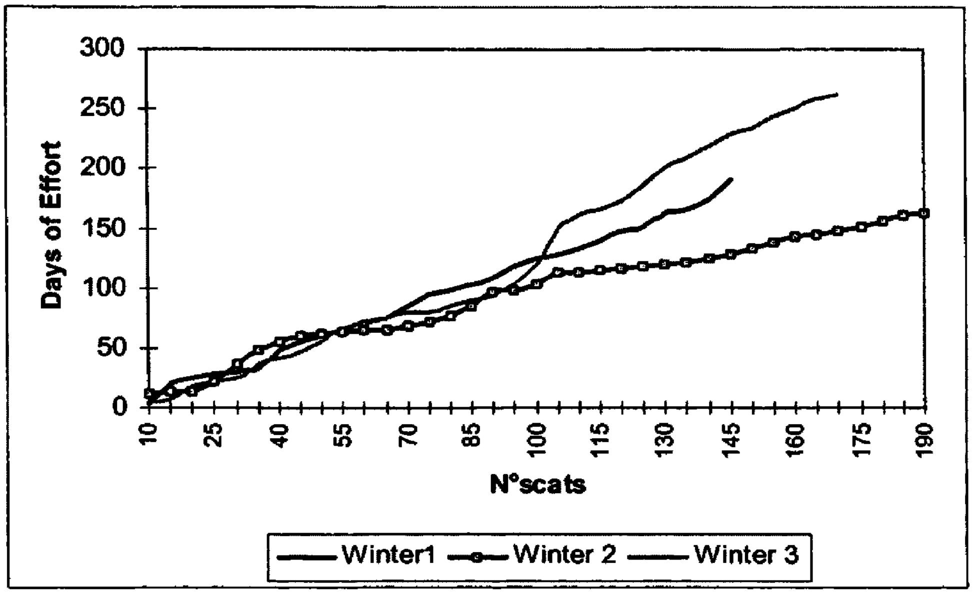


Figure 2. Standard error of the occurrence of each prey species in the wolf diet as a function of sample size of scats for winter 2000, the southwestern Alps of Italy and France.

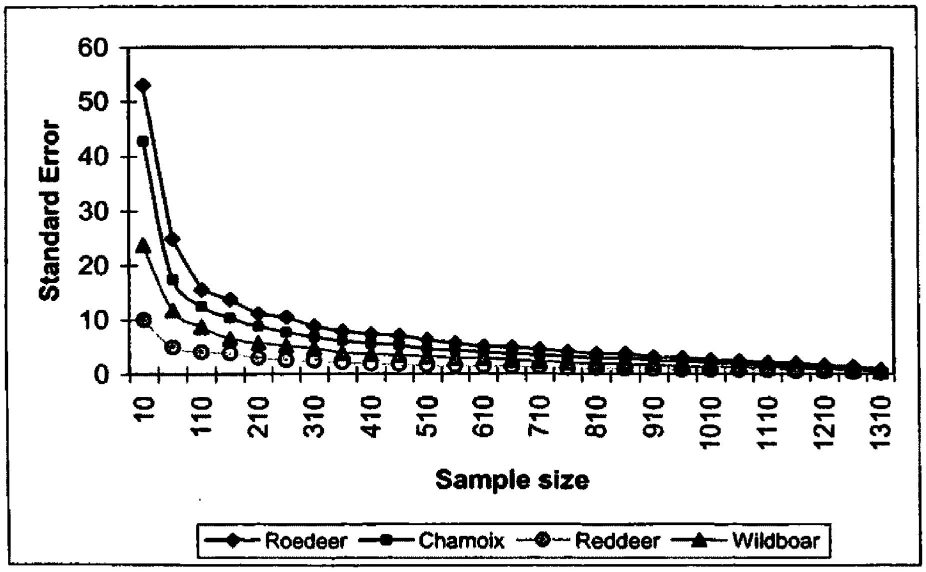


Figure 3. Standard error of the occurrence of each prey species in the wolf diet as a function of sample size of scats for winter 2001, the southwestern Alps of Italy and France.

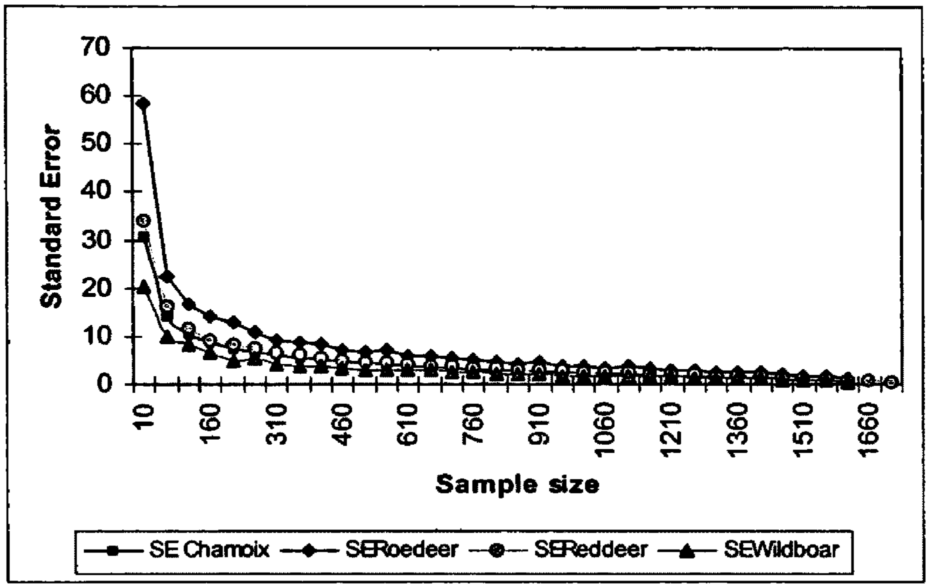
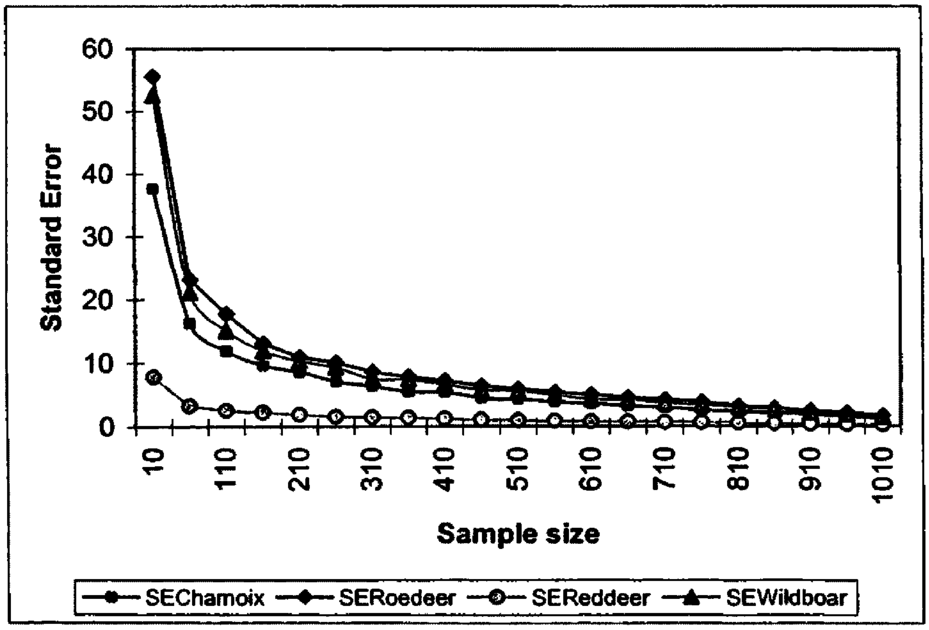


Figure 4. Standard error of the occurrence of each prey species in the wolf diet as a function of sample size of scats for winter 2002, the southwestern Alps of Italy and France.



DISCUSSION

Wolf Diet Selection

Wolves feed mostly on ungulates throughout the year both in North America (Fritts and Mech 1981, Potvin et al. 1988, Huggard 1993b, Marquard-Petersen 1998) and in Europe (Smietana and Klimek 1993, Ciucci 1994, Papageorgiou et al. 1994, Okarma 1995, Okarma et al. 1995). The importance of fruits, rubbish, and small mammals in some southern European areas where large herbivores are not abundant has been related to impoverished ecological situations (Boitani 1982, Reig and Jedrzejewski 1988, Meriggi et al. 1991). Poulle et al. (1995a,b) conducted the first food ecology study on wolves in the French Alps and demonstrated that wolves rely primarily on wild ungulates for prey.

Wild ungulates represented the bulk of the wolf's winter diet of the Valle Pesio pack. Although capturing larger prey requires increased effort, more food is secured when a kill is made (Marquard-Petersen 1998).

Roe deer composed the majority of the wolf's diet during each winter and they were the most important prey for wolves in other European countries as well (Jedrzejewski et al. 1992, Okarma 1995, Okarma et al. 1995). Deer are likely the most vulnerable species during severe winters (Jedrzejewski et al. 1992, Jedrzejewski et al. 2002). Red deer represented a small proportion of the Valle Pesio wolf pack winter diet, but were less abundant in the study area than other ungulate species. They were present only in the Valle Pesio proper (30-40 individuals). Despite their low availability, red deer were used more during the severe winter of 2001 when the larger wolf pack was present (6-8 individuals). Okarma et al. (1995) documented a significant positive correlation between the pack size and the number of red deer killed in Poland.

Wolves rarely fed on wild boar during winters 2000 and 2001. Wild boar was used only at the beginning of these 2 winters, and use was likely related to the human hunting period, when wolves could easily find injured or dead wild boars in the area (Ciucci 1994). However, a greater use of wild boar characterized winter 2002. Wild boar was the most important prey species for wolves in central Italy (Ciucci 1994, Mattioli et al. 1995, Guj 1996), whereas in other European countries with large cervid populations this species seemed to be avoided (Jedrzejewski et al. 1992, Okarma 1995).

Chamois were rarely used despite their high abundance in the study area. Chamois are an alpine species and well adapted to the snow conditions and rugged topography of the study area (Poulle and Lonchampt 1997), making them a difficult prey for wolves. Chamois were not abundant along wolf travel routes, and this is likely due to the lack of habitat overlap. Wolves do not select for steep and rocky terrain along their travel routes (Marucco 2001).

I evaluated availability along wolf travel routes and I did not consider the process of selection in the overall study area, but only within areas already selected by wolves. This measure of prey availability has never been tested and is likely biased according to the detectability of wolf tracks. For example, roe deer may have been over represented in the availability evaluation because it was easier to find wolf travel routes at the bottom of the valleys where roe deer occurred. However, during the snow cover period, the cervids tend to stay at lower altitudes where encounters with wolves are more likely. In these conditions, wolves can often travel from one predictable hunting territory to another and hunt intensively in these areas (Huggard 1993a, b). These spatial complexities may result in encounter rates for prey species that are not directly proportional to their densities (Huggard 1993b).

The scavenging behavior of wolves has been documented in North America (Frenzel 1974, Potvin et al. 1988, Huggard 1993c). I documented 4 cases of scavenging and found different food caching events during the 3 winter seasons. I noticed a particular searching behavior of wolves during late winter, when they intensively and systematically investigated avalanche areas. However, it was not possible to differentiate with scat analysis between scavenged and killed prey (Ciucci 1994), therefore prey use and not necessarily predation has been discussed.

Domestic ungulates, mainly goats and sheep, appeared in the winter wolf diet as a consequence of livestock depredations during the summer. The grazing period lasts from May-June to September-October in the Alps, therefore no livestock were available for the wolves during the winter season. The presence of livestock in the winter diet could be the result of "food caching" (Mech 1970).

Wolf depredations on livestock are an important issue in Europe (Brangi et al. 1991, Meriggi and Lovari 1996, Ciucci and Boitani 1998, Fourli 1999, Vos 2000) and now in the Alps (Espuno 2000, Poulle et al. 2000). Brangi et al. (1991) assumed that livestock are an alternative prey where wild ungulates are not abundant. In northern Portugal, the dependence of wolves on livestock can be explained by the scarcity of wild prey and the high density of livestock (Vos 2000). In the Alps, summer depredations on domestic ungulates are frequent (Tropini 2001) despite the presence of abundant wild ungulates. According to optimal foraging and optimal diet theories, wolves should select domestic prey because of their clumped distribution in localized pasture areas, low ability to recognize predators, and low escape effectiveness (Meriggi and Lovari 1996). However, the winter wolf diet shows that wolves can totally rely on wild ungulates in our study area when livestock are not available. Efficient measures discouraging wolves from taking domestic animals (e.g. use of guarding dogs, presence of a shepherd, nocturnal housing, etc.) might encourage year-round reliance on wild ungulate by wolves.

The interaction of wolves and their prey has been widely studied (Gasaway et al. 1983, Ballard et al. 1987, Gasaway et al. 1992, Huggard 1993c, Okarma 1995, Peterson 1995, Ballard et al. 1997, Kunkel 1997, Bergerud and Elliott 1998, Jedrzejewski et al. 2000); however, the dynamics of systems involving wolves and multiple prey remain poorly understood (Huggard 1993b). Prey availability and vulnerability are important factors in selection of prey (Gotelli 1998). Other factors such as snow cover (Huggard 1993b), wolf group size (Schmidt and Mech 1997), and availability of alternative prey (Dale et al. 1995), can also play an important role in predator-prey dynamics. When these factors vary between areas, a difference in the diet of wolves is likely to exist (Marquard-Petersen 1998). In the southwestern Alps, adjacent packs had very different diets. In the Valle Stura pack, approximately 20 km from the Valle Pesio pack, chamois constituted 80% of the wolf diet (Ricci 2001), while the nearby Mercantour pack selected moufflon (*Ovis gmelini*) (Poulle et al. 1995a). It is impossible to generalize wolf diets in the southwestern Alps, and this is likely due to the high diversity of prey species and densities across territories. Scat analysis is useful as a basic description of wolf's diet, for inter-pack comparisons of diet, and determining changes in diet with long term data bases. A long term study is important to document among-year and pack differences in scat content (Mills 1996).

Sampling Design For Scat Collection

Strong inference can be achieved with an accurate sampling design, reducing bias first and then increasing precision. Randomization reduces bias, whereas a low sampling variance, which can be achieved with large sample sizes or with a small population variance, increases precision, but it does not affect the bias (Manly 1996).

The worst situation is a biased and precise study design, where errors are hard to detect and results easy to trust because of their precision.

Bias and precision have been addressed at different steps of scat analysis and data analysis (Reynolds and Aebischer 1991, Ciucci et al. 1996), while few studies on scat analysis describe the procedure used to collect scats.

Bias

Potential for bias and misleading conclusions associated with scat collection include the design of field sampling, non-wolf scat inclusion in the analysis, and the independence of the data.

Organization of field sampling

Estimates may be biased because of non-random sampling (Manly 1996). Random sampling is often difficult to carry out, and there is a temptation to assume that a sample obtained in some convenient or arbitrary way is equivalent to a random sample. However, it is very easy for a systematic bias in the sampling procedure to distort estimates of key parameters to such an extent that a study becomes quite worthless (Manly 1996). Potential biases in scat collection may occur if haphazard sampling along human trails produces an unrepresentative sample. In my study, diet evaluated from scats collected only along human trails were similar to diet evaluated from scats collected along wolf travel routes. Almost all the scats were found on trails, likely due to the strong marking behavior of wolves (Asa et al. 1985, Vila et al. 1994). Searching for scats only along trails may not cause bias in the sampling design, but sampling should be randomly distributed along trails. Searching randomly for wolf tracks may allow us to indirectly assume a random collection of scats. However, the assumption that a random sample is obtained just because there is an element of randomness used in the selection does not hold (Manly 1996).

Randomization tests (Monte Carlo simulations or bootstrapping) could deal with the analysis of these types of samples.

Potential biases may occur also if sampling scats at rendezvous or kill sites causes overrepresentation of a prey species in the diet. Composition of scats from den or rendezvous sites can be significantly different from scats collected during equivalent periods along logging road and trails (Scott and Schackleton 1980, Marquard-Petersen 1998). Theberge et al. (1978) found higher proportions of beaver (*Castor canadensis*) in scats from rendezvous sites compared with collections from other areas used by wolves in Algonquin Park. Clusters of scat collected at a kill site can then overestimate that prey species in the diet. Mattson et al. (1991) sub-sampled scats where more than five were found at one kill site. The kill site issue was not important in my study because the wolf pack spent little time there, having no time to defecate the just eaten meal. Wolves soon left the kill because the majority of the kills were small (roe deer) and humans frequently disturbed wolves. This issue may be more important in other study areas where wolves spend more time at a kill site, and it should always be considered.

Non-wolf scats inclusion in the diet analysis

In most field conditions, no single criterion allows distinction between scats of wolves and other canids (Mattson et al. 1991, Litvaitis et al. 1996). A conservative, multi-criteria approach to differentiate wolf scats from those of other canids is usually used (Ciucci 1994, Poulle et al. 1995a). However, possible biases due to including non-wolf scats or due to discarding some wolf scats may occur. I detected only one non-wolf scat, from a fox, using genetic analysis on 150 scat samples. The fox scat was found at a kill site with wild boar hairs, but it didn't affect the diet estimation.

Scat collection in the summer may be highly affected by this problem, because wolf scats are hard to distinguish from dog scats (most shepherds have several dogs).

Independence

A basic assumption when determining diet by scat analysis is that each occurrence of a prey species in a scat represents an independent sample (Carss and Parkinson 1996). Scats collected on the same day and along the same travel route often contain hair from the same kill and are not independent samples (Huggard 1993b). They can belong to individuals of the same pack that eat together on the same carcass, or they might belong to the same individual. The same wolf could produce more than one scat, with the same content from the same meal, along one travel route. In these cases, scats were not independent; therefore, I considered a collection of scats and not a single scat as a sampling unit to avoid pseudo-replication (Hurlbert 1984) and met the assumption of independence, fundamental for selection studies (Thomas and Taylor 1990).

The "Additive Method"

Contents of scats collected along a wolf travel route can be expected to be representative of the carcass previously fed upon, if that carcass has not been found (Huggard 1993b). The additive data of carcasses and scat collections seemed to be more representative of the actual situation. Chamois was under-represented from only wolf-kill analysis; this apparent avoidance of chamois might be an artifact of the difficulty in finding chamois carcasses. Jedrzejewski et al. (2002) recommended this method in studies of wolf predation in dense woodlands and in regions where wolves consume small and medium sized ungulates (roe deer, piglets of wild boar). However, this method may still underestimate small prey species if ≥ 2 prey of the same species, killed and consumed by wolves in a short time sequence, are counted as one prey if only recovered from scat collections.

Precision

Defining clearly the population to be studied is an essential first step in planning a sample survey. Studies should be designed so that the correspondence between the target and the sampled population is as close as possible (Manly 1996); inference scope is therefore limited by the sampling design. A sample of scats that is small compared with the total number of scats produced may be unrepresentative (Reynolds and Aebischer 1991). Standard errors in assessing any aspect of the diet depend on the variability between scats and on the size of the sample (Reynolds and Aebischer 1991).

Different researchers argued that they had a large wolf scat sample, without relating it to the real scat population (e.g. Potvin et al. 1988, Poulle et al. 1995a). The real scat population is related to the number of wolves present in a pack, on the number of packs monitored, on the defecation rate of wolves, and on the time period considered. Potvin et al. (1988) argued that their sample of 1166 scats was large, but they did not specify on how many packs and wolves they were working. A sample is large or small only in relation to the real population size, not because it is a large or small number. For example, a sample of 300 scats from only 1 wolf in 1 year is a large sample, because it samples almost half of the scat population (assuming 2 scats/wolf/day). Instead a sample of 1000 scats from 4 wolf packs in 1 year is a small and perhaps not a representative sample, especially where diets between packs can differ greatly. It is fundamental that the results of similar studies should be comparable; therefore for quantitative data on scat composition it is desirable to quote the sample size relative to the estimated real scat population (%), and also the non-simultaneous bootstrap 95-percentile range for each food type together with the sample mean (Reynolds and Aebischer 1991).

Choice of an appropriate sample size is an essential component of any experimental design. I compared the power of various sample sizes and the relationship was a decreasing asymptotic function approaching zero. At small scat sample sizes, slight increases in sample sizes caused large reductions in the standard error, while at large scat sample sizes, further increases in sample size did not greatly affect the standard error. In this study the minimum acceptable sample size should be greater than 10-13% of the scat population. Sample sizes meeting this criterion did not differ greatly in resolving power and were likely to detect the presence of each prey species in the winter diet for the Valle Pesio wolf pack.

Monte Carlo simulations – the Method

I used a form of Monte Carlo simulation (Snedecor and Cochran 1980) to generate the standard error function, and to determine the sample size necessary for a chosen level of accuracy.

The Monte Carlo procedure is a computer-intensive statistic, which allows estimating the standard error of a statistic, using repeated samples from the original data set (Manly 1998). Bootstrapping is done by sampling with replacement to get samples of the same size as the original data set; therefore, it considers an infinite population. To deal with a finite population, as a scat population of a wolf pack, I had slightly better accuracy by estimating the standard error using repeated samples from the original data set without replacement, using a form of Monte Carlo simulation (Manly 1996). With samples from the highly non-normal distribution that are often encountered in biological studies, the method has the potential to be the most useful available approach for deciding on sample sizes (Manly 1998).

The limitation of the Monte Carlo approach is associated with drawing a range of sample sizes from a fixed pool size (n samples). The number of combinations for a given sample size varies for different sample sizes; in particular the function of possible combinations versus sample size is bell-shaped with the maximum number of combinations occurring at n/2 (Bros and Cowell 1987, Manly 1998). The bias introduced by this precludes the examination of changes in sample precision for sample sizes > n/2; therefore in my study samples > 700 for winter 2000, > 900 for winter 2001, and > 600 for winter 2002, must be considered carefully. However, for large sample sizes, further increases in sample size do not greatly affect the standard error. This limitation could be overcome by sampling the pilot sample with replacement rather then without replacement, using the bootstrap method (Manly 1998). I should then consider a finite population as an infinite population, overestimating the standard error because bootstrapping confidence intervals are wider than Monte Carlo ones. Therefore, I used the Monte Carlo procedure taking into account the possible bias introduced in assessing samples up to half the size of the total sample.

Optimization of the sampling design

Sampling designs are always constrained by the amount of time, personnel, materials, and money available for the project and therefore the feasibility should be considered (Bros and Cowell 1987). I identified the combination of factors that placed the greatest constraints on scat sample size, and in particular the time we spent to find and follow wolf tracks in the winter to find scats along wolf travel routes. By evaluating trade-offs between resolving power and expended effort, I evaluated that to have good precision in detecting the presence of each prey species in the diet in a winter for the Valle Pesio wolf pack, I needed at least an effort of 250 days of work in a winter. To detect the difference in the diet between packs (or years) one needs to evaluate the sample sizes necessary for a specified level of reliability; and to detect small changes in the diet a large scat sample size is required.

A huge effort is required to increase the scat sample size, and considering that often scat samples are not independent, it is possible that the increased sample size would not even improve the diet representation. Wolf diet studies should avoid pseudo-replication during scat sample collection, both along wolf travel routes, and at kill sites or rendezvous sites.

The additive method might reduce the scat sample size required for a representative sample because it adds kills data to the wolf diet evaluation. The additive method is optimal in diet selection studies because it deals with the independence issue, which is fundamental for selection studies (Thomas and Taylor 1990) and it allows one to estimate the standard error of the Manly's index of selection (1974).

The simultaneous examination of sampling design, power, and sampling effort allowed evaluating the costs and benefits of increasing the number of samples beyond the minimum acceptable size. By using this procedure in other studies on wolf diets, it is possible to evaluate the trade-offs between increasing sample size and increasing effort, depending on wolf pack size and a specific research question. This procedure provides a tool for optimizing sampling design in long-term studies on wolf diet.

LITERATURE CITED

Asa, C. S., L. D. Mech, and U. S. Seal. 1985. The use of urine, faeces, and anal-gland secretions in scent-marking by a captive wolf (Canis lupus) pack. Animal Behavior **33**:1034-1036.

Ballard, W. B., L. A. Ayres, P. R. Krausman, D. J. Reed, and S. G. Fancy. 1997. Ecology of wolves in relation to a migratory caribou herd in Northwest Alaska. Wildlife Monographs **135**:1-49.

Ballard, W. B., J. S. Whitman, and C. L. Gardner. 1987. Ecology of an exploited wolf population in south-central Alaska. Wildlife Monographs **98**:75-81.

Bergerud, T. A., and J. P. Elliott. 1998. Wolf predation in a multiple-ungulate system in nirthern British Columbia. Canadian Journal of Zoology **76**:1551-1569.

Boitani, L. 1982. Wolf management in intensively used areas of Italy. Pages 158-172 in F. H. Harrington, and P. C. Paquet, editors. Wolves of the world. Perspectives of behavior, ecology and conservation. Noyes publications, Park Ridge, New Jersey.

Boyd, D., R. Ream, D. H. Pletscher, and M. W. Fairchild. 1994. Prey taken by colonizing wolves and hunters in the Glacier National Park area. Journal of Wildlife Management **58**:289-295.

Brangi, A., P. Rosa, and A. Meriggi. 1991. Predation by wolves (Canis lupus L.) on wild and domestic Ungulates in Northern Italy. Pages 541-543. Ongules / Ungulates.

Bros, W. E., and B. C. Cowell. 1987. A technique for optimizing sample size (replication). J. Exp. Mar. Biol. Ecol. **114**:63-71.

Carss, D. N., and S. G. Parkinson. 1996. Errors associated with otter *Lutra Lutra* faecal analysis. I. Assessing janeral diet from spraints. J. Zool. **238**:301-317.

Chesson, J. 1978. Measuring preference in selective predation. Ecology **59**:211-215.

Ciucci, P. 1994. Movimenti, attività e risorse del lupo (Canis lupus) in due aree dell'Appennino centro-settentrionale. Page 117. University of Roma "La Sapienza", Roma, Italy.

Ciucci, P., and L. Boitani. 1998. Wolf and dog depredation on livestock in central Italy. Wildlife Society Bulletin **26**:504-514.

Ciucci, P., L. Boitani, E. R. Pelliccioni, M. Rocco, and I. Guj. 1996. A comparison of scat-analysis methods to assess the diet of the wolf Canis lupus. Wildl. Biol. **2**:37-48.

Ciucci, P., S. D'Alessio, L. Mattei, and L. Boitani. 2001. Stima della biomassa consumata tramite analisi degli escrementi: calibrazione del modello sulle principali prede selvatiche del Lupo in Italia. III Congresso Italiano di Teriologia, San Remo, Italia.

Dale, B. W., L. D. Adams, and R. T. Bowyer. 1995. Winter wolf predation in a multiple ungulate prey system in L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and conservation of wolves in a changing world. Canadian Circumpolar Institute, Edmonton, Alberta, Canada.

Espuno, N. 2000. Effect of herd management practices on wolf predation on livestock in the Mercantour mountains, France. Page 85. Beyond 2000. Realities of Global Wolf Restoration, Duluth, Minnesota, USA.

Floyd, T. J., L. D. Mech, and P. A. Jordan. 1978. Relating wolf scat content to prey consumed. Journal of Wildlife Management **43**:528-532.

Fourli, M. 1999. Compensation for damage caused by bears and wolves in the European union. Pages 1-68. Life-Nature Projects, Bruxelles.

Frenzel, L. D. 1974. Occurrence in food in moose of wolves as revealed by scat analysis: a review of North American studies. Nat. Can. **101**:467-479.

Fritts, S. H., and L. D. Mech. 1981. Dynamics, movements, and feeding ecology of a newly protected wolf population in Northwestern Minnesota. Wildlife Monographs **80**:1-78.

Gasaway, W. C., R. D. Boertje, D. V. Grangaard, D. G. Kelleyhouse, R. O. Stephenson, and D. G. Larsen. 1992. The role of predation in limiting moose at low densities in Alaska and Yukon and implications for conservation. Wildlife Monographs **120**:1-59.

Gasaway, W. C., R. O. Stephenson, J. L. Davis, P. E. K. Shepherd, and O. E. Burris. 1983. Inter-relationships of wolves, prey and man in interior Alaska. Wildlife Monographs **84**:1-50.

Gotelli, N. J. 1998. A primer of ecology. Sinauer Associates, Inc., Sunderland, Massachusetts.

Guj, I. 1996. Ecologia alimentare del lupo (Canis lupus L.) in un'area dell'Appennino settentrionale. Pages 1-144. University of Roma, Roma.

Huggard, D. J. 1993a. Effects of snow depth on predation and scavenging by gray wolves. Journal of Wildlife Management **57**:382-388.

Huggard, D. J. 1993b. Prey selectivity of wolves in Banff National Park. I. Prey Species. Can. J. Zool. **71**:130-139.

Huggard, D. J. 1993c. Prey selectivity of wolves in Banff National Park. II. Age, sex, and condition of elk. Canadian Journal of Zoology **71**:140-147.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs **54**:187-211.

Jedrzejewski, W., B. Jedrzejewski, H. Okarma, and A. L. Ruprecht. 1992. Wolf predation and snow cover as mortality factors in the ungulate community of the Bialowieza National Park, Poland. Oecologia **90**:27-36.

Jedrzejewski, W., B. Jedrzejewski, H. Okarma, K. Schmidt, K. Zub, and M. Musiani. 2000. Prey selection and predation by wolves in Bialowieza Primeval Forest, Poland. Journal of Mammology **81**:197-212.

Jedrzejewski, W., K. Schmidt, J. Theuerkauf, B. Jedrzejewski, N. Selva, K. Zub, and L. Szymura. 2002. Kill rates and predation by wolves on ungulate populations in Bialowieza Primeval Forest (Poland). Ecology **83**:1341-1356.

Krebs, C. J. 1998. Ecological methodology. Addison-Welsey Educational, Menlo Park.

Kunkel, K. E. 1997. Predation by wolves and other large carnivores in northwestern Montana and southeastern British Columbia. Page 272. University of Montana, Missoula.

Kunkel, K. E., T. K. Ruth, D. H. Pletscher, and M. G. Hornocker. 1999. Winter prey selection by wolves and cougars in and near Glacier National Park, Montana. Journal of Wildlife Management **63**:901-910.

Litvaitis, J. A. 2000. Investigating food habits of terrestrial vertebrates in L. Boitani, and T. K. Fuller, editors. Research techniques in animal ecology. Controversies and consequences. Columbia University Press, New York.

Litvaitis, J. A., K. Titus, and E. M. Anderson. 1996. Measuring vertebrate use of terrestrial habitats and foods. Page 740 in T. A. Bookhout, editor. Research and management techniques for wildlife and habitats. The Wildlife Society, Bethesda, Maryland.

Manly, B. F. J. 1974. A model for certain types of selection experiments. Biometrics **30**:281-294.

Manly, B. F. J. 1996. The design and analysis of research studies. Cambridge University Press, New York.

Manly, B. F. J. 1998. Randomization, Bootstrap and Monte Carlo Methods in Biology. Chapman & Hall, London.

Manly, B. F. J., L. L. McDonald, and D. L. Thomas 1993. Resource selection by animals: statistical design and analysis for field studies. Chapman & Hall, New York, USA.

Marquard-Petersen, U. 1998. Food habits of arctic wolves in Greenland. Journal of Mammology **79**:236-244.

Marucco, F. 2001. Monitoraggio e ricerca sulla popolazione di lupo: lo studio intensivo nelle Alpi Liguri. Pages 63-134. Regione Piemonte - Interreg II Italia-Francia, Torino.

Mattioli, L., M. Apollonio, V. Mazzarone, and E. Centofanti. 1995. Wolf food habits and wild ungulate availability in the Foreste Casentinesi National Park, Italy. Acta Theriologica **40**:387-402.

Mattson, D. J., B. M. Blanchard, and R. R. Knight. 1991. Food habits of Yellowstone grizzly bears, 1977-1987. Canadian Journal of Zoology **69**:1619-1629.

Mech, L. D. 1970. The wolf. The ecology and behavior of an endangered species. University of Minnesota Press, Minneapolis.

Meriggi, A., and S. Lovari. 1996. A review of wolf predation in southern Europe: does the wolf prefer wild prey to livestock? Journal of Applied Ecology **33**:1561-1571.

Meriggi, A., P. Rosa, A. Brangi, and C. Matteucci. 1991. Habitat use and diet of the wolf in northern Italy. Acta Theriologica **36**:141-151.

Mills, M. G. L. 1996. Methodological advances in capture, census, and food habits studies on large african carnivores. Pages 223-242 in J. L. Gittleman, editor. Carnivore Behavior, Ecology, and Evolution. Comstock Publishing Associates, London.

Okarma, H. 1995. The trophic ecology of wolves and their predatory role in ungulate communities of forest ecosystems in Europe. Acta Theriologica **40**:335-386.

Okarma, H., B. Jedrzejewski, W. Jedrzejewski, Z. A. Krasinski, and L. Milkowski. 1995. The roles of predation, snow cover, acorn crop, and man-related factors on ungulate mortality in Bialowieza Primeval Forest, Poland. Acta Theriologica **40**:197-217.

Papageorgiou, N., C. Vlachos, A. Sfougaris, and E. Tsachalidis. 1994. Status and diet of wolves in Greece. Acta Theriologica **39**:411-416.

Perco, F. 1986. Ungulati. Lorenzini, Udine.

Peterson, R. O. 1995. The wolves of Isle Royale: a broken balance. Willow Creek, Minocqua, WI.

Potvin, F., H. Jolicoeur, and J. Huot. 1988. Wolf diet and prey selectivity during two periods for deer in Quebec: decline versus expansion. Can. J. Zool. **66**:1274-1279.

Poulle, M.-L., L. Carles, and B. Lequette. 1995a. Significance of ungulates in the diet of recently settled wolves in the Mercantour mountains (southeastern France). Rev. Ecol. (Terre Vie) **52**:357-368.

Poulle, M.-L., T. Houard, and T. Dahier. 1995b. Le suivi des loups dans le Parc National du Mercantour. Bulletin mensuel de l'Office National de la Chasse **201**:36-45.

Poulle, M.-L., and F. Lonchampt. 1997. Regime alimentaire des loups dans le Mercantour. Faune de Provence **18**:33-40.

Poulle, M.-L., T. Dahier, R. Beaufort, and C. Durand. 2000. Le loup en France. Pages 1-90. Projet Life-Nature.

Randi, E., V. Lucchini, M. F. Christensen, N. Mucci, S. M. Funk, G. Dolf, and V. Loeschckes. 2000. Mitochondrial DNA variability in Italian and East European wolf: detecting the consequences of small population size and hybridazion. Conservation Biology **14**:1-11.

Reig, S., and W. Jedrzejewski. 1988. Winter and early spring food of some carnivores in the Bialowieza National Park, eastern Poland. Acta Theriologica **33**:57-65.

Reynolds, J. C., and N. J. Aebischer. 1991. Comparison and quantification of carnivore diet by faecal analysis: a critique, with recommendations, based on a study of the Fox Vulpes vulpes. Mammal. Rev. **21**:97-122.

Ricci, S. 2001. Monitoraggio e ricerca sulla popolazione di lupo: lo studio estensivo in Provincia di Cuneo. Pages 11-62. Regione Piemonte - Interreg II Italia-Francia, Torino.

Schmidt, P. A., and L. D. Mech. 1997. Wolf pack size and food acquisition. The American Naturalist **150**:513-517.

Scott, B. M. V., and D. M. Schackleton. 1980. Food habits of two Vancouver Island wolf packs: a preliminary study. Can. J. Zool. **58**:1203-1207.

Smietana, W., and A. Klimek. 1993. Diet of wolves in the Bieszczady Mountains, Poland. Acta Theriologica **38**:245-251.

Snedecor, G. W., and W. G. Cochran 1980. Statistical Methods. Iowa State University Press, Ames.

Theberge, J. B., S. M. Oosenbrug, D. H. and Pimlott. 1978. Site and seasonal variations in food of wolves, Algonquin Park, Ontario. Canadian Field-Naturalist. **92** (1): 91-94.

Thomas, D. L., and R. J. Taylor. 1990. Study designs and tests for comparing resource use and availability. Journal of Wildlife Management **54**:322-330.

Tosoni, E. 2002. Il lupo nel Parco Nazionale del Pollino: ecologia, metodi di quantificazione e strategia di campionamento della dieta. Pages 1-195. Animal Biology. University of Roma, La Sapienza, Roma.

Tropini, A. 2001. Monitoraggio e valutazione dei danni al patrimonio zootecnico in Provincia di Cuneo. Pages 234-250. Regione Piemonte, Torino.

Vila, C., V. Urios, and J. Castroviejo. 1994. Use of faeces for scent marking in Iberian wolves (Canis lupus). Canadian Journal of Zoology **72**:374-377.

Vos, J. 2000. Food habits and livestock depredation of two Iberian wolf packs (*Canis lupus signatus*) in the north of Portugal. J.Zool. **251**:457-462.

Weaver, J. L. 1993. Refining the equation for interpreting prey occurrence in grey wolf scats. Journal of Wildlife Management **57**:534-538.

Chapter 4. Extended Conclusion

Estimating the density and monitoring population trends of large carnivores is one of the most difficult tasks that a wildlife biologist or manager can undertake. In some cases accurate methods exist, but they require large amounts of fieldwork, high costs, and invasive methods like radio-collaring animals (Quigley and Hornocker 1992, Linnell et al. 1998). Large carnivores, such as wolves, have low densities and are very hard to observe and detect because they are often nocturnal and occupy dense habitats (Linnell et al. 1998). Large carnivore populations generally have slow growth rates (Quigley and Hornocker 1992); therefore a failure to detect a real decline in population abundance can be serious (Taylor and Gerrodette 1993). Decades may be needed for the population to recover. Because of these problems, many different methods have been used to estimate the abundance of large carnivore populations and to monitor their distribution (Lancia et al. 1994). Because of advantages and constraints within various habitats types, species-specific methods have been developed.

I used, discussed, and evaluated limitations of the non-invasive methods that seem promising in the context of the European Alps. Before a monitoring program for a particular species can be designed and implemented, the objective of the monitoring program has to be clearly stated (Goldsmith 1991). I followed the dynamics and the diet selection of one wolf pack over 3 years (Chapters 2 and 3) to test and calibrate the non-invasive methods that can be applied over a larger area. These methods, applied in the same manner on other wolf packs, could help document population trends. However, these methods don't allow us to understand the complexity of the process of population changes. Radiotelemetry is needed, along with the non-invasive techniques, to investigate important phenomena such as dispersal, mortality, habitat selection, and kill rates.

Long-term studies are important for developing conservation and management strategies. A good sampling design with the best cost-benefit approach is fundamental for pursuing this aim. Data on the wolf recolonization process may dispel misperceptions and thereby direct management decisions that help wolves to survive in a fragmented and human-dominated habitat such as the Alps.

In a European context, where wolf packs have transboundary territories (Marucco 2001, Ricci 2001, Apollonio 2001, Duchamp et al. 2002), it is difficult to have only one coordinated wolf management plan that covers the different countries (Italy, France, and Switzerland). Despite the Convention of Bern (11.19.1979), and the Convention on International Trade in Endangered Species (CITES; 3.3.1973), which consider the wolf a fully protected species in all the European Alps countries, Switzerland has implemented a management plan which allows harvesting wolves under special conditions (KORA, date unknown). France is in a "changing" management situation, where harvesting by zones is being considered. Italy is still guaranteeing full protection to wolves although poaching is still widely practiced (Boitani and Ciucci 1993).

An accurate research approach and population viability analyses are needed in this heterogeneous Alps context. Large carnivore management requires careful monitoring, considering the ability of hunters and poachers to reduce these populations to the edge of extinction (Swenson et al. 1994, Breintenmoser 1998). Effective quota setting (determination of maximum allowable mortality) in a management plan can be achieved if precise population estimates exist and population dynamics are understood such that the harvestable proportion of the population can be calculated (Linnell et al. 1998). In this context, effective refuge areas with no harvest and guard programs that reduce poaching could be important as potential source areas for wolves repopulating the adjacent lands. In fact, refuge areas have been advocated on a theoretical basis in recent years (McCullough 1996). The problem in Europe is that refuges are always too small for most large carnivores that have large home ranges and low densities. Therefore, a long-term wolf research project, evaluating wolf population and predator-prey dynamics in the Alps, is fundamental, and it should consider the diverse political situations and the heterogeneous land use to guide European management decisions.

LITERATURE CITED

Apollonio, M., P. Bertotto, I. Bertelli, A. Gazzola, M. Scandura, L. Mauri, and S. Luccarini. 2001. Lo studio estensivo in Provincia di Torino e Valle Po. Pages 135-188. Regione Piemonte - Interreg II Italia-Francia, Torino.

Boitani, L., and P. Ciucci. 1993. Wolves in Italy: critical issues for their conservation. Pages 74-90 in C. Promberger, and W. Schroder, editors. Wolves in Europe. Status and perspectives. Munich Wildlife Society, Ettal, Germany.

Breitenmoser, U. 1998. Large predators in the Alps: the fall and rise of man's competitors. Biological Conservation **83**:279-289.

Duchamp, C., T. Dahier, and B. Lequette. 2002. Compte rendu du suivi hivernal des loups dans l'arc alpin francais: bilan de l'hiver 2001/2002. Pages 1-13. Programme Life Nature.

Goldsmith, F. B. 1991. Monitoring for conservation and ecology. Chapman and Hall, London.

KORA. Date unknown. Documentazione Lupo redatta su mandato dell'Ufficio federale dell'ambiente delle foreste e del paesaggio (UFAFP). www.kora.unibe.ch/pdf/docus/docwf_i.pdf.

Lancia, R.A., J.D. Nichols, and K.H. Pollock. 1994. Estimating the number of animals in wildlife populations. Pages 215-253 in Research and Management Techniques for Wildlife and Habitats. Fifth ed. The Wildlife Society, Bethesda, MD.

Linnell, J. D. C., J. E. Swenson, A. Landa, and T. Kvam. 1998. Methods for monitoring European large carnivores. A worldwide review of relevant experience. Pages 1-38. NINA - Norsk institutt for naturforskning, Trondheim.

Marucco, F. 2001. Monitoraggio e ricerca sulla popolazione di lupo: lo studio intensivo nelle Alpi Liguri. Pages 63-134. Regione Piemonte - Interreg II Italia-Francia, Torino.

McCullough, D. R. 1996. Spatially structured populations and harvest theory. Journal of Wildlife Management **60**:1-9.

Quigley, H. B., and M. G. Hornocker. 1992. Large carnivore ecology: from where do we come and to where shall we go? Pages 1089-1097 in Wildlife 2001: Populations. Ed. D. R. McCullough and R. H. Barrett.

Ricci, S. 2001. Monitoraggio e ricerca sulla popolazione di lupo: lo studio estensivo in Provincia di Cuneo. Pages 11-62. Regione Piemonte - Interreg II Italia-Francia, Torino.

Swenson, J. E., F. Sandegren, A. Bjarvall, A. Soderberg, P. Wabakken, and R. Franzen. 1994. Size, trend, distribution and conservation of the brown bear *Ursus arctos* population in Sweden. Biological Conservation **70**:9-17.

Taylor, B.L., and T. Gerrodette. 1993. The uses of statistical power in conversation biology: the vaquita and northern spotted owl. Conservation Biology 7:489-500.

APPENDIX A

Laboratory analysis

1. Laboratory analysis

I followed the laboratory procedures of Reynolds and Aebischer (1991). Prior to treatment, I thawed, oven-dried (90°C for 6 h) and weighed each scat to the nearest 0.01 g. Next, I soaked each scat in water for 24-48 h and separated micro from macro-components of each scat by washing it in a sieve with a mesh size of 0.5 mm. The microscopic fraction of the scat is represented by water-soluble particles and components fragmented finely enough to pass through the sieve, whereas all other remains, larger than the mesh size, represent the macro-components that I further identified. I discarded the microscopic fraction based on the assumption that it originates from food items in the same proportions as the macroscopic remains (Ciucci et al. 1996). I separated macro-components for each scat by food items (e.g. hairs, bones, seeds) and I estimated their relative volume proportion with the aid of a superimposed reference grid (Reynolds and Aebischer 1991). Each scat was completely broken apart (no clumps of hairs were left) and at least 30 hairs were randomly selected from each scat on the grid for examination, to avoid overlooking of situations were > 1 prey item was in the scat (Spaulding et al. 2000). I excluded from the analysis items believed to be ingested unintentionally, i.e. leaves, soil, and rumen (Ciucci et al. 1996). I did not include in the analysis items when they composed < 3% of the scats because these were likely remains of a previous meal.

I identified mammalian hairs by microscopic examination of the cuticular pattern, the medulla, and the cross-section and then compared these samples to hairs of mammals collected locally or to photos shown in a reference manual (Teerink

1991). I identified bone, nail, and teeth remains by referencing to museum specimens.

2. Measuring the accuracy of identification (Blind test)

Scat analyses are conducted in laboratories and prey species are identified by microscopic examinations (Reynolds and Aebischer 1991, Ciucci 1994, Marquard-Petersen 1998, Jedrzejewski et al. 2000). Biases can occur and precision can be affected by observer ability in the identification of prey remains (Fritts and Mech 1981, Ciucci et al. 1996). The accuracy and the consistency of the method across years can be increased by a pre-sampling training period which measures the level of accuracy and minimizes the different abilities of observers in identifying prey species (Fritts and Mech 1981).

In a pre-sampling training period I assessed accuracy of observers (Francesca Marucco, Tommaso Galli, Eglantine Aubin, and Luca Orlando) in identifying mammal hairs through a blind test on a sample of 120 hairs from local mammals (Fritts and Mech 1981). The mean accuracy for the 4 observers in the item identification, evaluated through a blind test, was 98.7%. In particular one observer had an accuracy of 100%, and the other three of 98.3%. I considered this an acceptable level of accuracy (Ciucci 1994). The blind test also minimized the different abilities between observers to identify prey species; therefore this pre-sampling training period was useful to have a consistent methodology.

3. Cross-identification to test consistency between observers

In a post-identification period I assessed consistency of observers (Francesca Marucco, Tommaso Galli and Eglantine Aubin) in identifying mammal hairs through a cross-test on a sub-sample of 90 scats from the total scat sample. Therefore, I tested consistency between observers through a cross-identification test. Two observers identified the same scat sample (n=30); therefore, a total of 90 random scats from the total scat sample, was cross-identified by the three observers.

The concordance between the observers was 93.3%.

4. Presence of more then one item in a scat

Spaulding et al. (2000) found that incomplete analysis of scats, and perpetuation of the assumption that wolf scats contain only 1 prey item/scat resulted in missing many of the microtines and birds; thus, incomplete dissection may under represent these species.

I conducted complete examination of each scat, and I dissected every clump of hair to find any smaller prey or second prey species that may be hidden within clumps of ungulate hair or in the center of a scat (Spaulding et al. 2000).

I detected 2 species of prey in 10.3% of the scats (n=435); only 1 scat contained 3 different prey species. I didn't find microtines or birds. Scats with > 1 prey species contained only ungulates.

LITERATURE CITED

Ciucci, P. 1994. Movimenti, attività e risorse del lupo (Canis lupus) in due aree dell'Appennino centro-settentrionale. Page 117. University of Roma "La Sapienza", Roma, Italy.

Ciucci, P., L. Boitani, E. R. Pelliccioni, M. Rocco, and I. Guj. 1996. A comparison of scat-analysis methods to assess the diet of the wolf Canis lupus. Wildl. Biol. 2:37-48.

Fritts, S. H., and L. D. Mech. 1981. Dynamics, movements, and feeding ecology of a newly protected wolf population in Northwestern Minnesota. Wildlife Monographs **80**:1-78.

Jedrzejewski, W., B. Jedrzejewski, H. Okarma, K. Schmidt, K. Zub, and M. Musiani. 2000. Prey selection and predation by wolves in Bialowieza Primeval Forest, Poland. Journal of Mammology **81**:197-212.

Marquard-Petersen, U. 1998. Food habits of arctic wolves in Greenland. Journal of Mammology **79**:236-244.

Reynolds, J. C., and N. J. Aebischer. 1991. Comparison and quantification of carnivore diet by faecal analysis: a critique, with recommendations, based on a study of the Fox Vulpes vulpes. Mammal. Rev. **21**:97-122.

Spaulding, R. L., P. R. Krausman, and W. B. Ballard. 2000. Observer bias and analysis of gray wolf diets from scats. Wildlife Society Bulletin **28**:947-950.

Teerink, B. J. 1991. Hairs of West European mammals. Cambridge University Press, Great Britain.

APPENDIX B

S-PLUS Program for Monte Carlo simulations

S-PLUS 6.1 program used in the Monte Carlo simulations for the precision analysis of the winter diet of the Valle Pesio wolf pack, southwestern Alps of Italy and France. This program was written by Scott Jhones, Department of Math, University of Montana.

```
sim.ci.ss<-function(X,s,reps,factor,alpha)
{## X is a data set with one miscellaneous column and exactly
## one other column of real valued covariate information.
## s should be a vector of sample sizes. factor is the number
## of times X should be replicated to approximate the population.
## alpha is the level to compute the monte carlo confidence intervals
## reps is the number of iterations used for making the confidence
## interval for each (s) value.
m<-length(s)
RESULTS<-array(rep(0,4*m),c(m,4))
i<-1
j<-1
while(i<=m)
{temp<-montecarlosim(X,s[i],reps,factor,alpha)$SUMMARIES
RESULTS[i,1]<-s[i]
RESULTS[i,2]<-temp[1,1]
RESULTS[i,3]<-temp[1,2]
RESULTS[i,4]<-temp[1,3]
i<-i+1}
plot(cbind(RESULTS[,1],RESULTS[,1]),cbind(RESULTS[,2],RESULTS[,4]),xlab="
Sample size",ylab="Confidence interval",type="n")
text(RESULTS[,1],RESULTS[,2],labels=rep("--",m))
text(RESULTS[,1],RESULTS[,4],labels=rep("--",m))
text(RESULTS[,1],RESULTS[,3],labels=rep("0",m))
return(RESULTS)}

generatepop<-function(X,factor){
## X is a data set, factor is an integer expressing the number
## of times you want to replicate the sample to approximate the
## original population. For example, if |X|=100 and you estimate
## the population size to be 500, then set factor=5
i<-1
j<-1
f<-1
n<-length(X[,1])
k<-length(X[1,])
Y<-array(rep(0,n*factor*k),c(n*factor,k))
while(f<=factor)
```

```
{i<-1
while(i<=n)
{j<-1
while(j<=k)
{Y[i+(f-1)*n,j]<-X[i,j]
j<-j+1}
i<-i+1}
f<-f+1}
return(Y)}

montecarlosample<-function(X,s)
{## X is a data set, s is the size of a sample taken without replacement
## from X. The sample is returned by the function.

n<-length(X[,1])
k<-length(X[1,])
Yindex<-sample(n,size=s,replace=F)
Y<-array(rep(0,s*k),c(s,k))
i<-1
j<-1
while(i<=s)
{j<-1
while(j<=k)
{Y[i,j]<-X[Yindex[i],j]
j<-j+1}
i<-i+1}
return(Y)}

montecarlosim<-function(X,s,reps,factor,alpha)
{## X is the data set, the first column of which is miscellaneous
## identification information. The rest of the columns are numeric
## real covariates, confidence intervals of which will be simulated
## using the Monte Carlo method. First, POP is created as a best
## guess of the original population. The population is (factor)
## replicates of the sample X. For (reps) iterations, a sample
## of size (s) is drawn without replacement from POP and the
## sample means of each covariate are recorded as a column in
## RECORDS. Finally, RECORDS is used to come up with
## non-simultaneous confidence intervals at the specified (alpha)
## level (ie, 1-alpha confidence intervals). A Summary is produced
## for each covariate, showing the mean accross monte carlo samples
## as well as the estimated lower and upper bounds of each
## confidence interval.
POP<-janeratepop(X,factor)
n<-length(POP[,1])
k<-length(POP[1,])
RECORDS<-array(rep(0,reps*k),c(reps,k))
SUMMARIES<-array(rep(0,3*(k-1)),c(k-1,3))
i<-1
j<-1
```

```
while(i<=reps)
{j<-1
temp<-montecarlosample(POP,s)
while(j<=k-1)
{RECORDS[i,1]<-i
RECORDS[i,j+1]<-mean(temp[,j+1])
j<-j+1}
i<-i+1}
i<-1
j<-1
while(i<=k-1)
{SUMMARIES[i,1]<-quantile(RECORDS[,i+1],alpha/2,na.rm=F)
SUMMARIES[i,2]<-mean(RECORDS[,i+1])
SUMMARIES[i,3]<-quantile(RECORDS[,i+1],1-alpha/2,na.rm=F)
i<-i+1}
XNAMES<-names(X)[-1]
YNAMES<-c("Lower bound","Mean","Upper bound")
return(RECORDS,XNAMES,YNAMES,SUMMARIES)}
```

APPENDIX C

Monte Carlo simulations

I evaluated the effect of scat sample size on the precision of estimating frequency of occurrence in the wolf diet of each wild ungulate prey species (Chamois, Roe deer, Red deer, Wild boar), during each winter (Winter 1999-2000 (1), Winter 2000-2001 (2), Winter 2001-2002 (3)) (Figure B1 a-n).

Figure C1 (a-n). Effect of scat sample on 95% confidence intervals of the frequency of occurrence of a wild ungulate prey species in wolf scats of the Valle Pesio wolf pack in the southwestern Alps, Italy and France. The vertical axis is the proportion of wolf scats containing prey species hairs, for samples of increasing size (horizontal axis). Monte Carlo CI were evaluated through 1000 simulated samples derived without replacement (equivalent to sampling from a finite population of 1510 for Winter 2000, 1790 for Winter 2001, and 1190 for Winter 2002).

Figure C1.a. Winter 2000 - Chamois

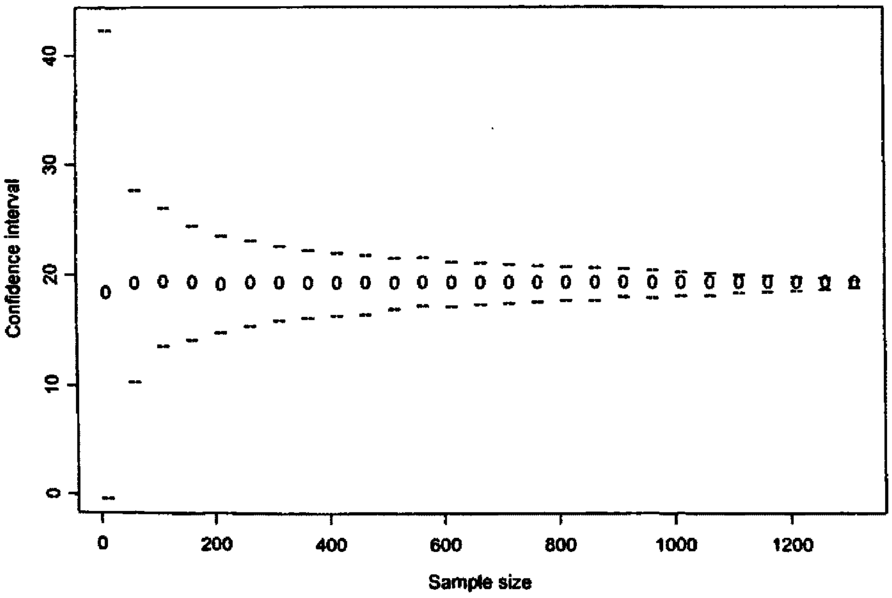


Figure C1.b. Winter 2000 – Roe deer

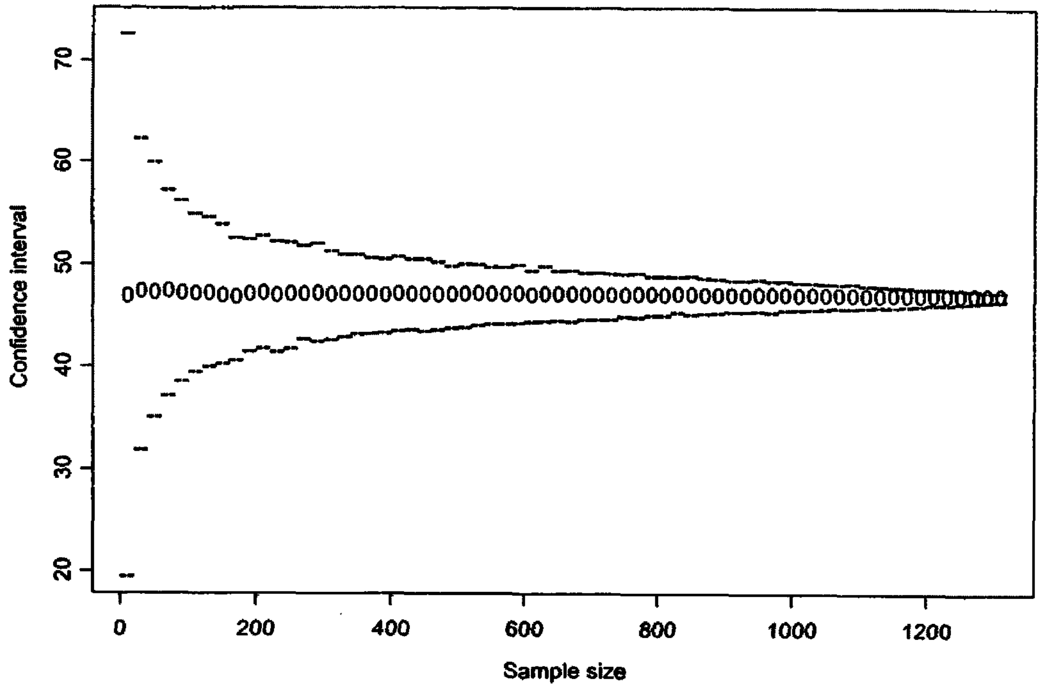


Figure C1.c. Winter 2000 - Red deer

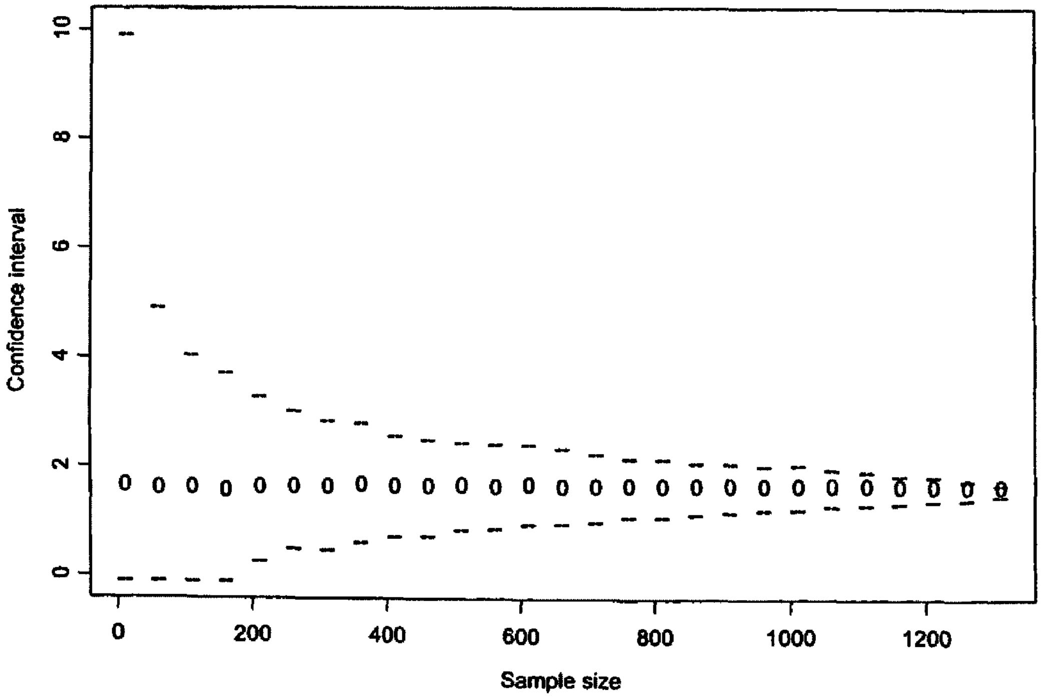


Figure C1.d. Winter 2000 - Wild boar

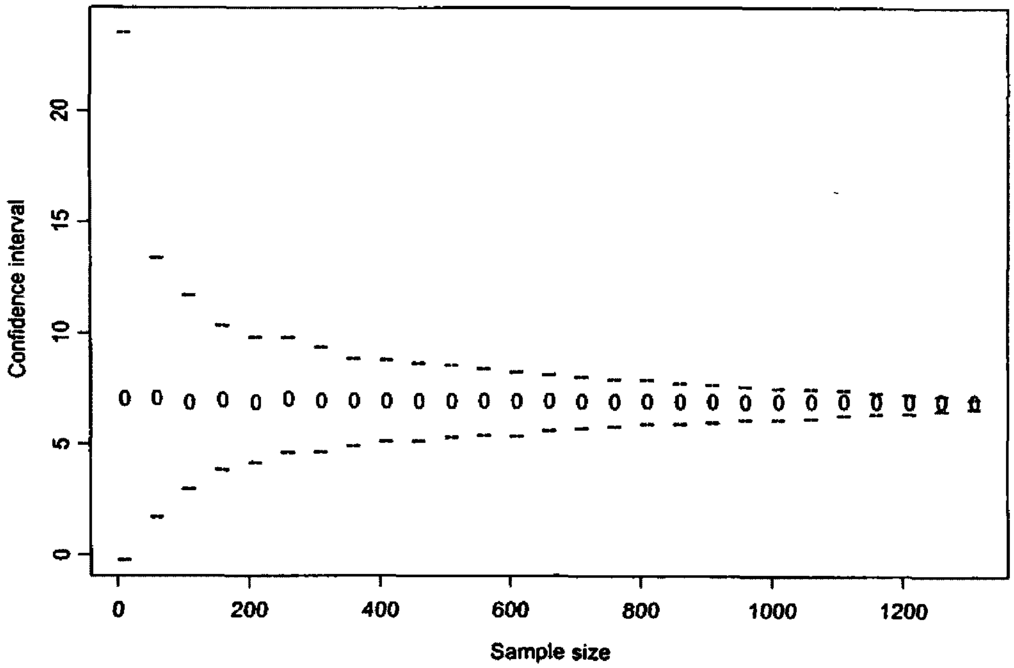


Figure C1.e. Winter 2001 - Chamois

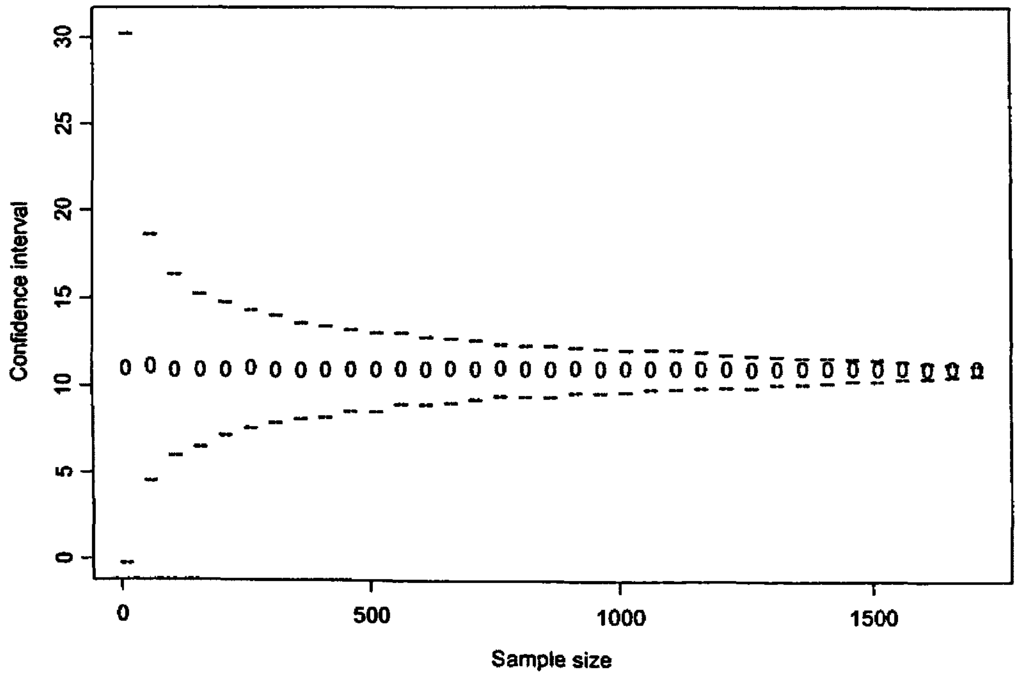


Figure C1.f. Winter 2001 - Roe deer

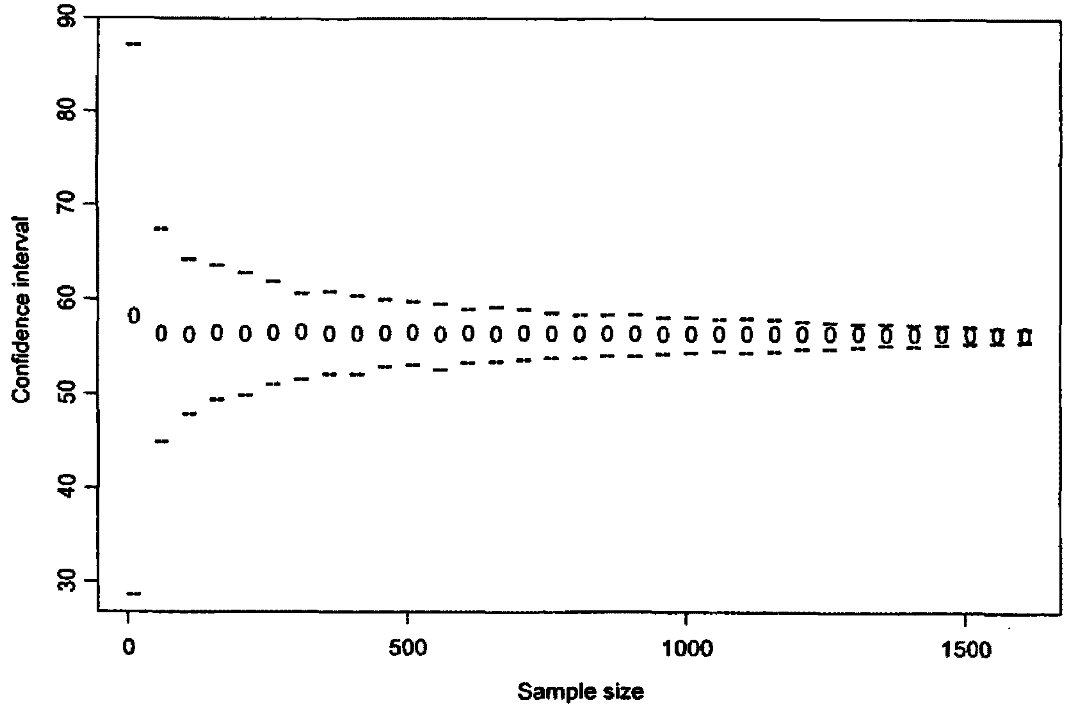


Figure C1.g. Winter 2001 - Red deer

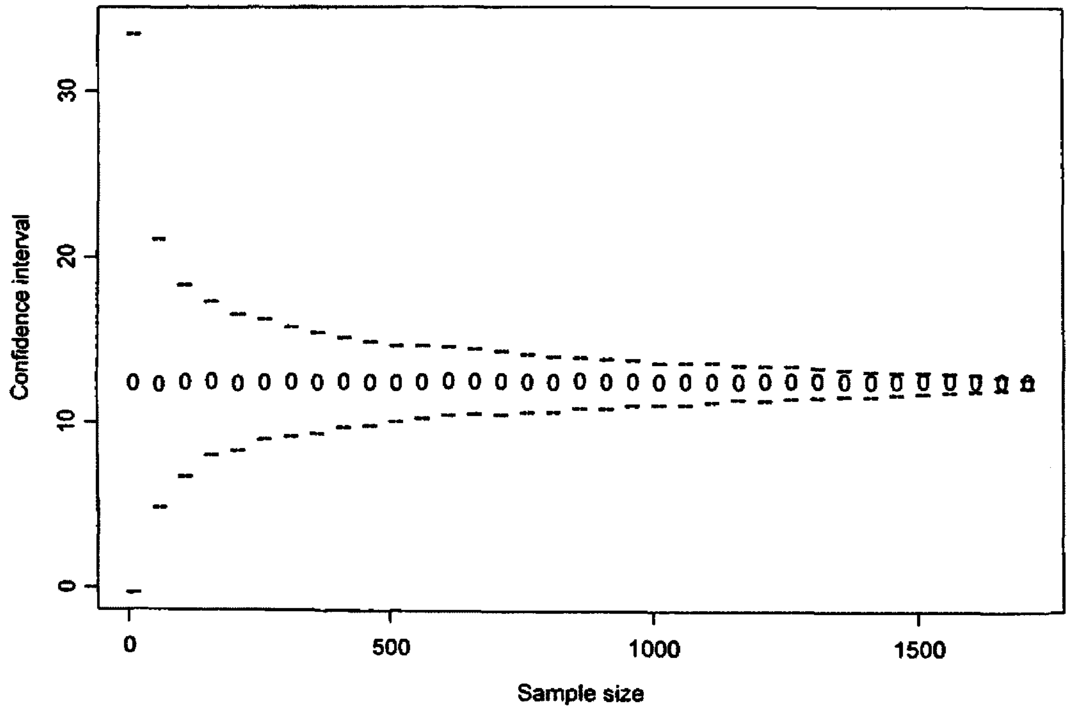


Figure C1.h. Winter 2001 - Wild boar

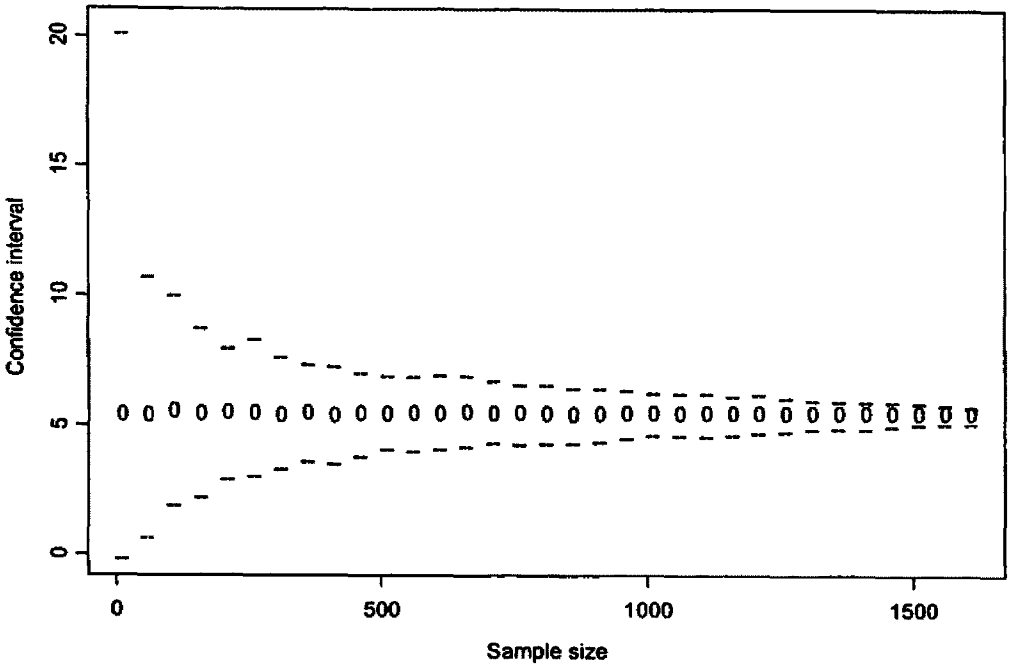


Figure C1.i. Winter 2002 - Chamois

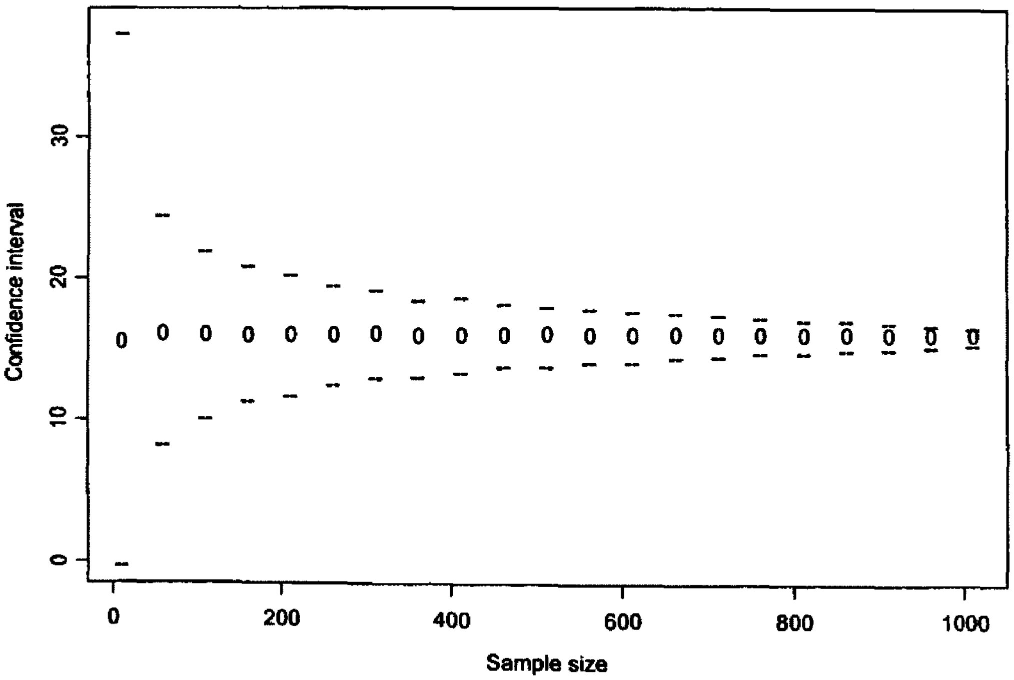


Figure C1.l. Winter 2002 - Roe deer

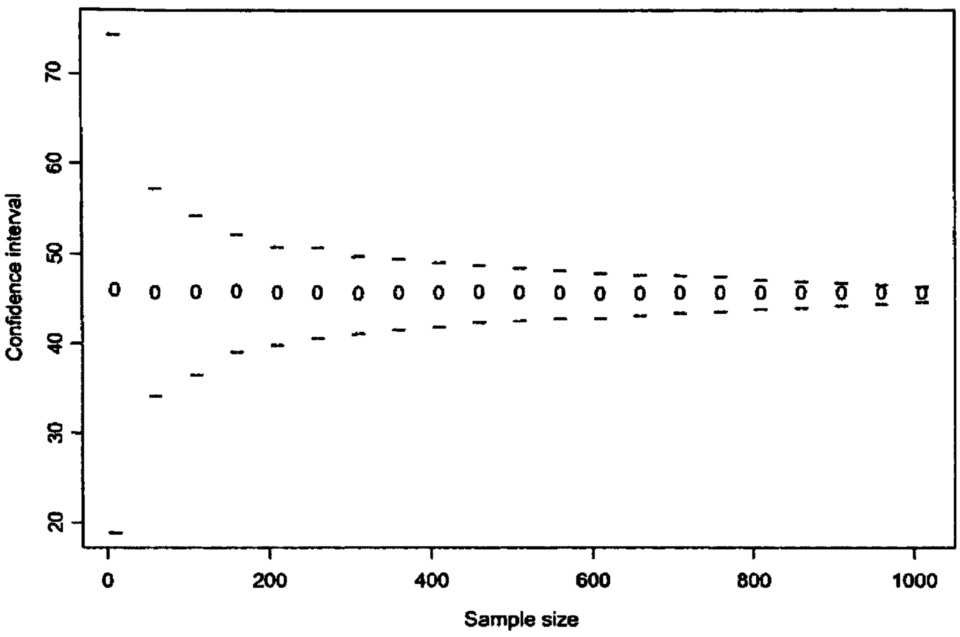


Figure C1.m. Winter 2002 - Red deer

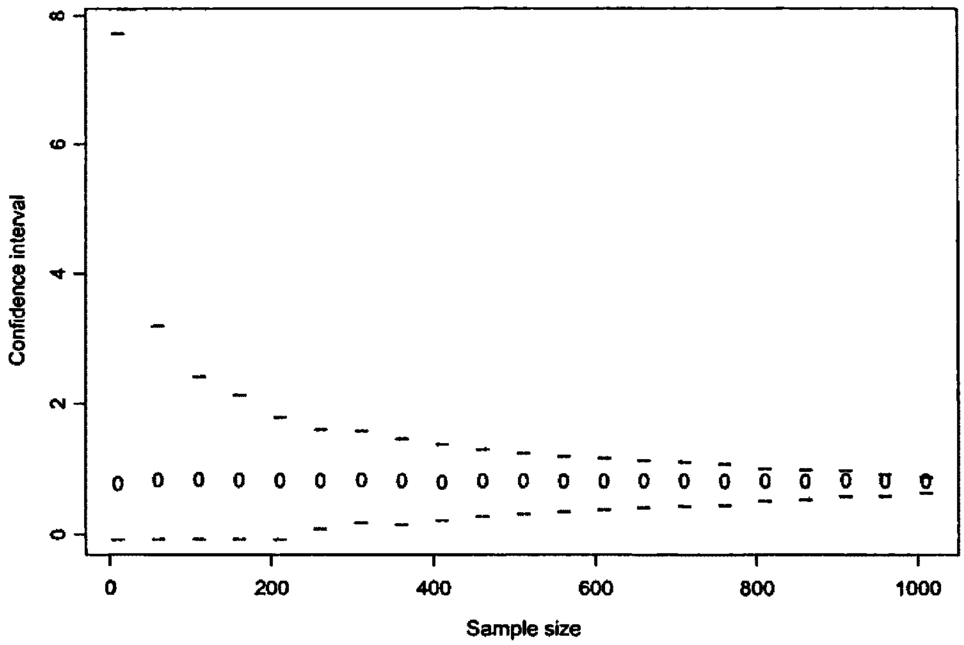


Figure C1.n. Winter 2002 - Wild boar