



*LIFE11 NAT/IT/069/ Med Wolf - Best practice actions for wolf
conservation in Mediterranean-type areas*

Assessment of wolf presence in expansions areas in Italy

Action D4

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with the contribution of

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1. INTRODUCTION

The monitoring of wolf presence and distribution in Grosseto province was carried out in 2017 in order to obtain an estimate of the population that could be also used as an indicator of project success.

The action started on 1/7/2016 and lasted until 30/11/2017.

The work was conducted under technical coordination from IEA while the scientific supervision was provided by the “Sapienza” University of Rome .

The field work was carried out by three technicians contracted by the province of Grosseto after public tender and selection.

Respect to what was foreseen in the project proposal we decided to cover a larger area, because the data available at the beginning of the project (the results of the LIFE IBRIWOLF project) did not allow to estimate the size and the distribution of wolf population over the provincial territory.

The main objectives of the survey were the following:

- Estimate the presence and distribution of the species;
- Estimate the wolf population size
- Provide adequate data to feed into the mapping of potential risk of human-wolf conflict (Action A6)

In this report we present the results of the survey, including a discussion of population estimation based on the application of mark-recaptures models of sampled genotypes, and the development of models of suitability and potential distribution of the species, which are reported in detail in the annexes.

The positive results obtained with the state-of-the-art methods we are confident that this work could be a model for surveying wolf presence elsewhere.

2. PROJECT AREA

The sampling area was selected in order to optimize resources and time available and in full consideration of the local socio-ecological conditions (e.g., wolf ecology, hunting activities). On the basis of the preliminary suitability model produced in Action A6 we selected 172 3x3km cells (1,548 Km²) which accounted for 48% of the highly suitable area for wolf in the Province (fig.1)

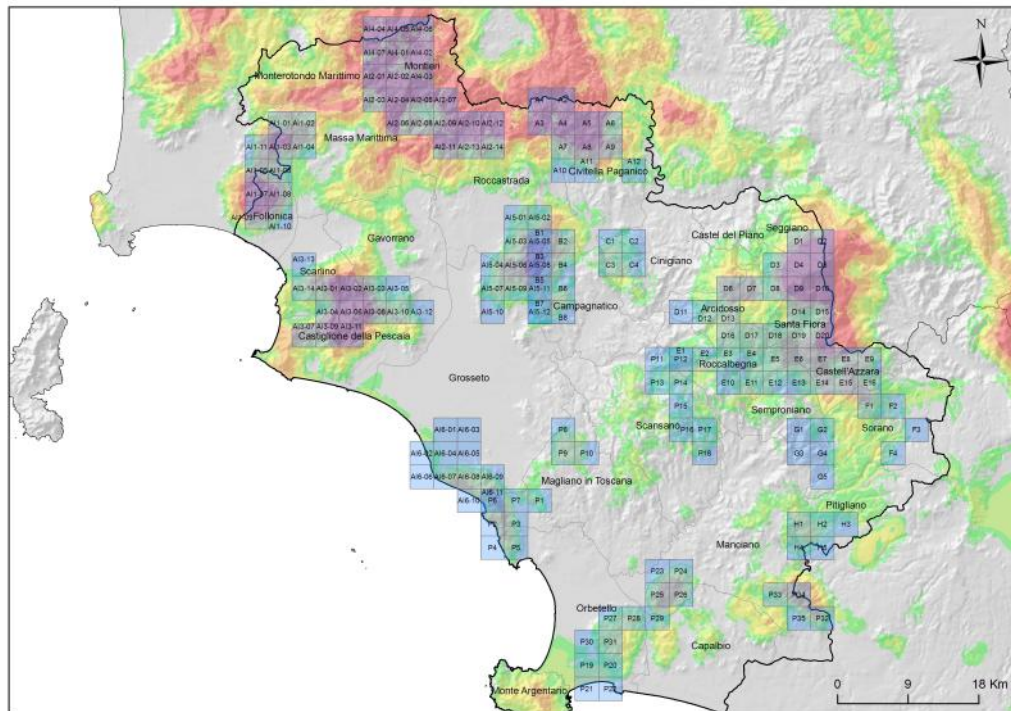


Figure 1. Sampling area for the wolf presence survey.

The sampling area was divided in 3 sectors: North, East, and South, and each sector was monitored by one technician.

3. METHODS

Wolf presence data were collected using the following research techniques:

- Scat collection for genetic analyses to estimate: population size, presence and distribution of the species
- Camera trapping to estimate: presence and distribution of the species, spatial organization, reproduction, pack size and composition and phenotypic characteristics of individuals
- Wolf howling to estimate: presence and distribution of the species, reproduction and spatial organization

3.1 Scats collection

Field work started in April 2017 and lasted until September of the same year. A pilot test to verify the applicability of the sampling design was performed in March.

The field work was organized in 3 different sessions: April, June and September. During these months an intensive effort to find wolf scats was made. In each cell we travelled by foot and by car in order to cover possible direction of movements of the animals. Once we found a scats we registered the coordinates, and on the basis of the estimated age of deposition we collected a sample for genetic analysis.

Collection was also made opportunistically while performing other survey activities, and in August during wolf-howling activities we made 4 attempts to collect scats at rendezvous sites.

The samples collected were analysed at the INBIO-CIBIO lab in Porto, Portugal, in order to ensure coherence with samples analysed within the LIFE IBRIWOLF project.

A set of 16 microsatellites were used to genotype the samples (the same microsatellites used during LIFE IBRIWOLF), which were selected based on genetic differentiation between wolf and dog.

3.2 Camera trapping

A total of 35 camera traps were used in the surveyed areas. The camera traps were opportunistically installed inside the sampling cells, where signs of presence of the species were registered in order to maximize the probability of a snapshot.

The activity of camera trapping was particularly intense during the months of May, July and September. Camera trap data obtained during the summer period was used to obtain confirmation of the reproduction events in the packs.

Each camera trap was installed for at least 15 days and then moved to another place depending also on the results obtained.

For each video/photo we recorded the following: number of animals, presence of pups, other sign of reproduction (e.g. signs of lactation) and phenotypic characteristics of individuals as a possible indication of hybridization with domestic dogs.

3.3 Wolf-howling

Wolf-howling was used to detect reproducing packs in the area. To achieve this objective we followed the protocol proposed by Harrington & Mech (1982) for “saturation census” approach. The area to be censused was defined on the basis of its suitability to host a rendezvous (RV) site. This was done considering 3 environmental variables: vegetation cover, distance to roads, and distance from human settlements.

A grid of lines at approximately 3-km intervals was overlapped to the study area and each intersection point of the grid represented a howling station. The exact station location was then modified, during specific field surveys, taking into account environmental factors that could influence sound transmission and possible human disturbance (fig.2).

Each station was censused on 3 consecutive nights. A single stimuli was used in order to reduce difference in reply rate between small and large packs (Harrington and Mech 1982).

Howling sessions started on the 5th of July 2017 and ended on the 31st of July of the same year.

Where we suspected the potential presence of adjacent packs, the neighbouring stations were covered simultaneously.

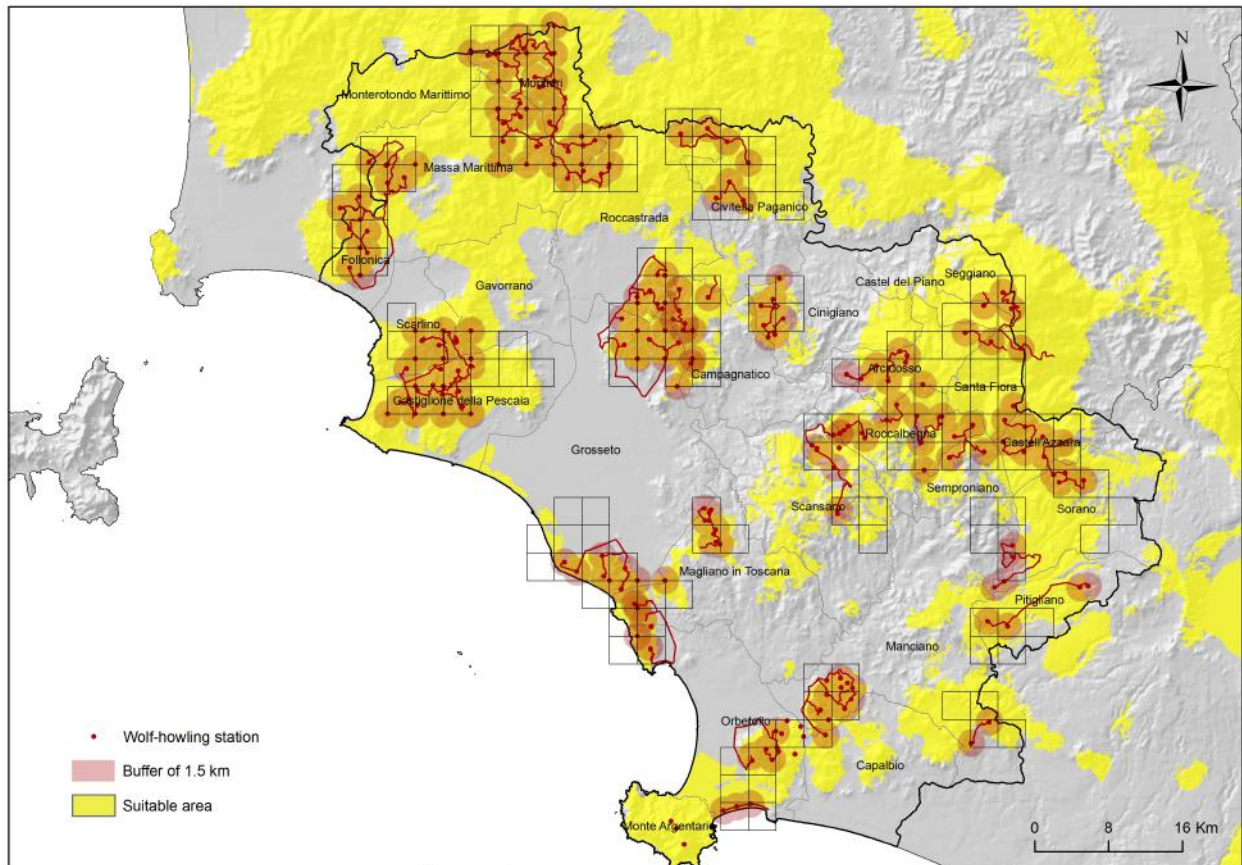


Figure 2. Distribution of wolf-howling stations.

While implementing the data collection sessions, we also recorded other signs of presence: prey remains, tracks, dead wolves

All collected data were recorded in a specific database divided by type of sign of presence of the species.

3.4 Data analysis

The presence data collected were used to build:

- An interpolated estimate of the wolf distribution in the province
- an estimate of the population present
- Human-wolf conflict risk map as per Action A6

For the population estimate, the data were analysed using two maximum likelihood-based modelling approaches. The first is the Schwarz and Arnason (1996) parametrization of the Jolly-Seber model (hereafter POPAN estimator) which includes immigration and emigration dynamics in the parameter structure. The second (Capwire, Miller et al. 2005) models a close population but it is robust to closure assumption violations. The models were implemented using respectively the packages Rmark (Laake 2013) and Capwire (Pennel & Miller 2012) in program R (R core team 2017).

A detailed description of the methodology used for habitat modelling and range interpolation and the results obtained is presented in the specific report (Annex 1 and report for Action A6). Here we only extracted a synthesis of the results in order to present all the activities carried out in the frame of this action.

4. RESULTS

4.1 Scat collection and genetic analysis

During the survey period (April-September 2017) 2,220 Km were covered to search for wolf scats. Scats were found in 112 different cells of the grid (fig. 3). A total of 978 scats were recorded and 289 were sent to the lab for genetic analysis.

DNA extraction was possible from 94% (n = 272) of samples. Over these 272 samples, based on the analysis of mitochondrial DNA 87,50% was attributed to the wolf while the remaining 12,50% to other species (red fox 6,62%, dog 4,04%, wild boar 1,47%, and marten 0,37%) This can be considered an estimate of the reliability of the operators, although in some cases the assignment to other species could be attributed to a possible contamination of the samples. It should be considered that 6 samples for which the mitochondrial DNA was of wild boar were genotyped as wolf at the level of nuclear DNA.

Overall 138 samples (46,6%) of the 289 sent to the lab were used for individual genotyping (132 samples resulted wolves or putative wolf x dog crosses and 6 dogs)

From April to September 2017 a total of 63 genotyped wolves or putative wolf x dog crosses were registered in the sampling area: 31 males and 32 females (fig. 4), and 57% of the genotypes were sampled only once while the remaining 43% between 2 to 8 times (fig. 5).

We genotyped also 5 other wolves from tissue analysis (2 dead wolves) and hair samples (2 dead wolves, and 1 was a sample found in a sheep fence after predation).

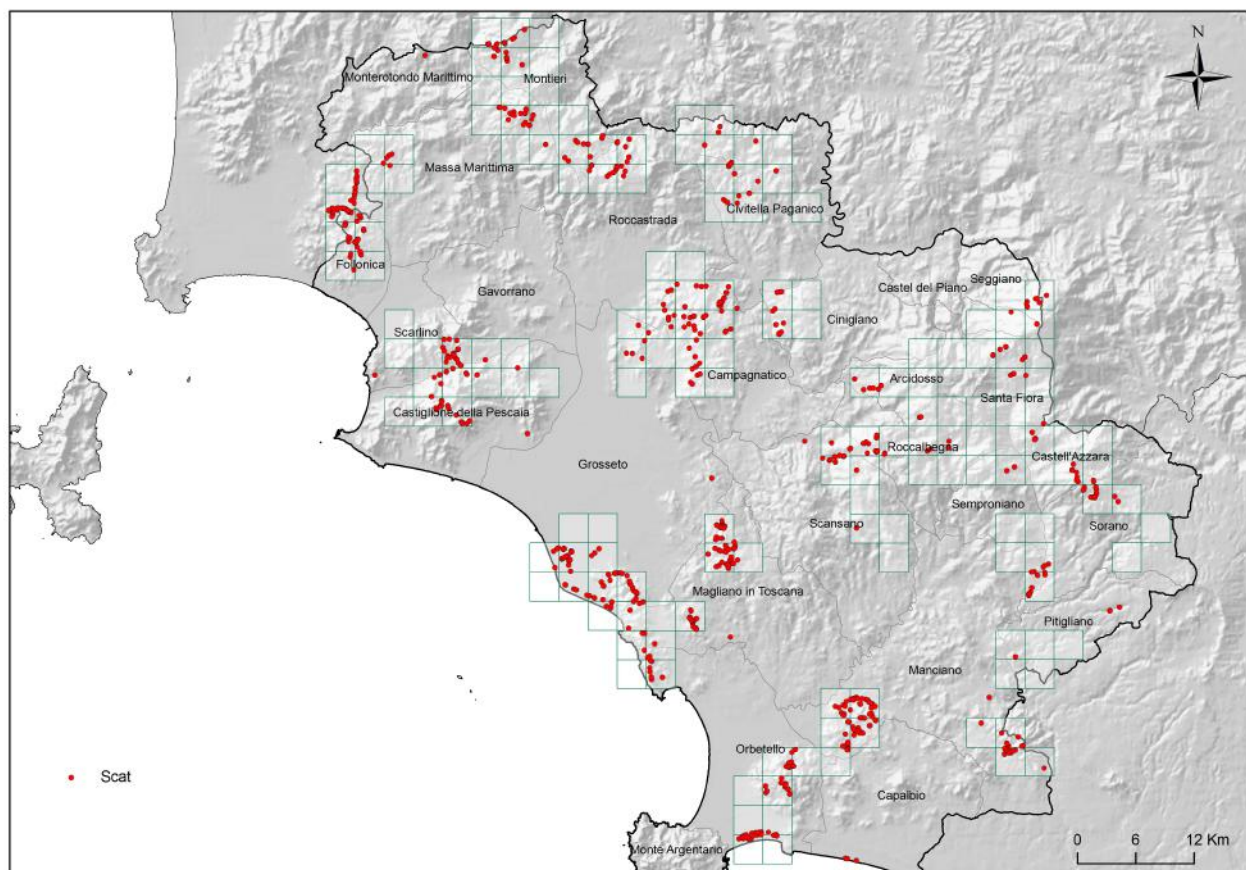


Figure 3. Distribution of scats recorded in the project area.

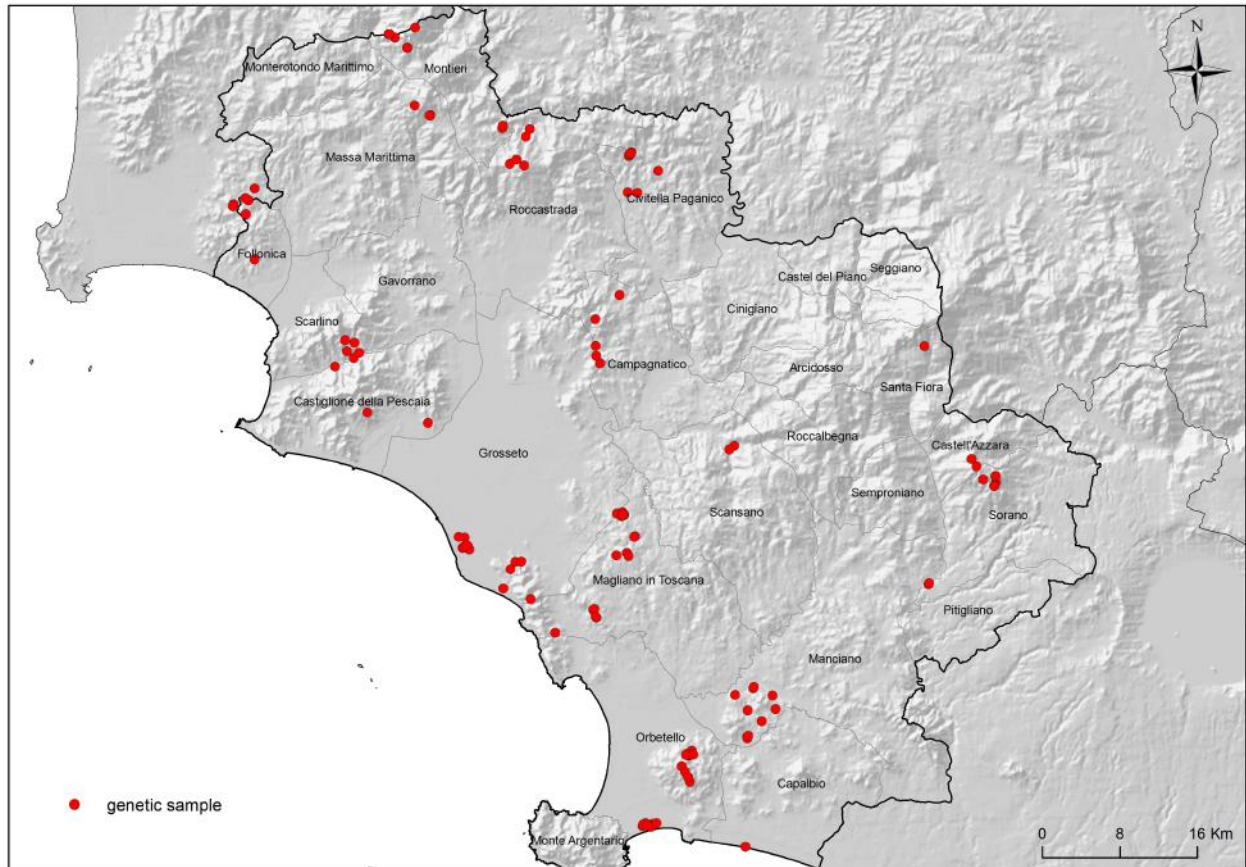


Figure 4. Distribution of genetic samples successfully genotyped.

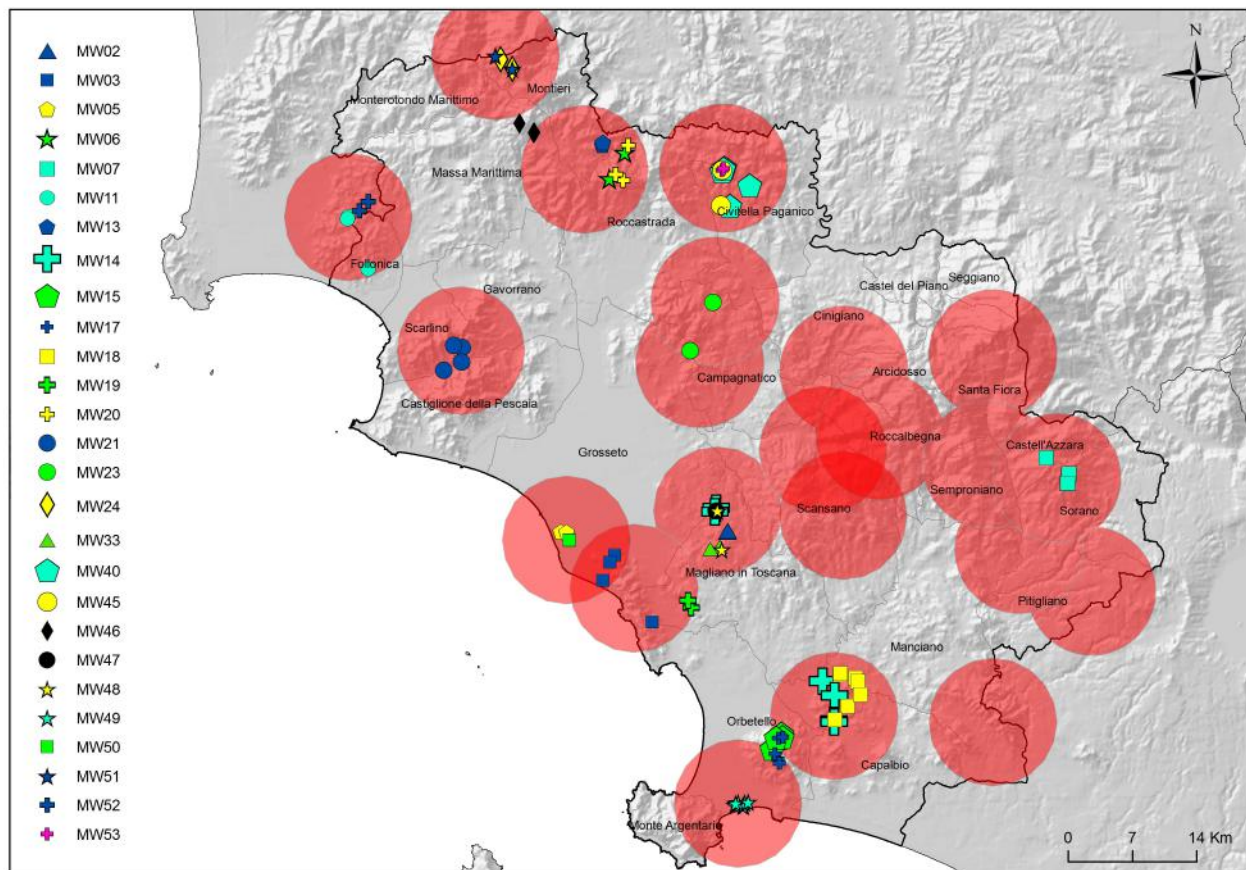


Figure 5. Distribution of genotypes sampled more than once.

4.2 Camera trapping

Videos and photos of wolves were obtained from camera traps placed in 43 different cells of the sampling grid (fig. 6). We obtained 744 videos/photos of wolves (2,356 trap/nights) (fig. 7), in this calculation we have also included animals with phenotypic characteristics attributable to wolf-dog hybrids on the basis of the morphological characters defined by Ciucci (2012).

The number of animals “captured” ranged from 1-8 and in 22% of the videos/photos there was at least 1 pup.

Camera trapping was very useful to determine the reproductive success of the packs. The reproduction of 6 packs was exclusively determined by camera trapping.

Even if a specific analysis of the phenotypic characters of animals sampled was not made (because out of the scope of the project) we registered different individuals with possible signs of hybridization across the study area.

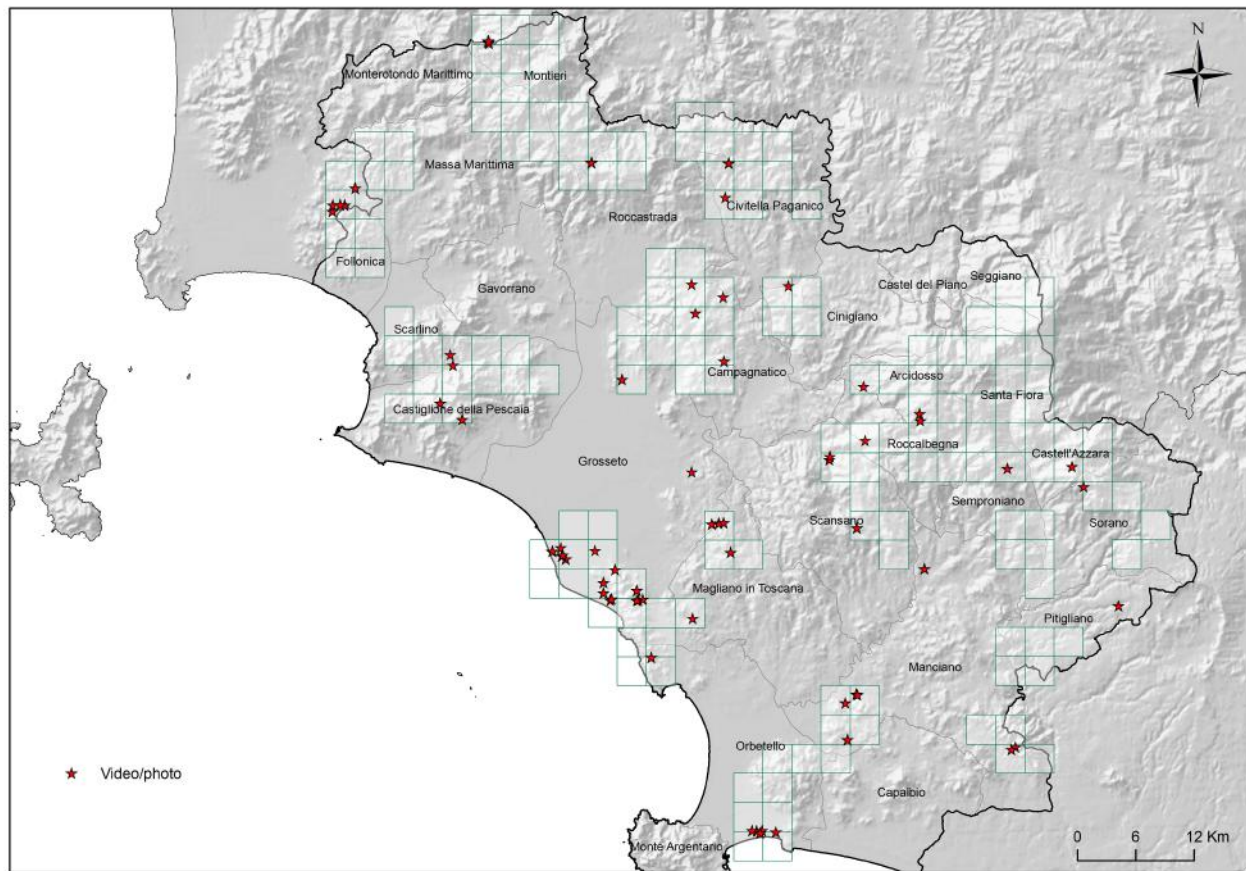


Figure 6. Distribution of videos/photos obtained by camera trapping



Figure 7. Wolf filmed with camera trap in the sampling area (M. Lucchesi).

4.3 Wolf-howling

In the period 5-31st of July 2017 we surveyed for 3 consecutive nights 19 different circuits with 136 howling stations. For each wolf reply we registered the location, the eventual presence of pups and the number of animals that replied.

When pups were heard replying we made additional howling sessions in August and September 2017 to better locate the possible RV site. This was done in order to attempt a specific pup scat collection in these areas.

Overall we obtained 37 wolf replies, 51% of which with puppies (this includes also 6 spontaneous howlings).

Based on the replies with pups, the simultaneity of the replies, and distance between replies we registered the presence of 10 reproducing packs.

Howling replies were obtained from 19 different cells of the sampling grid (fig. 8).

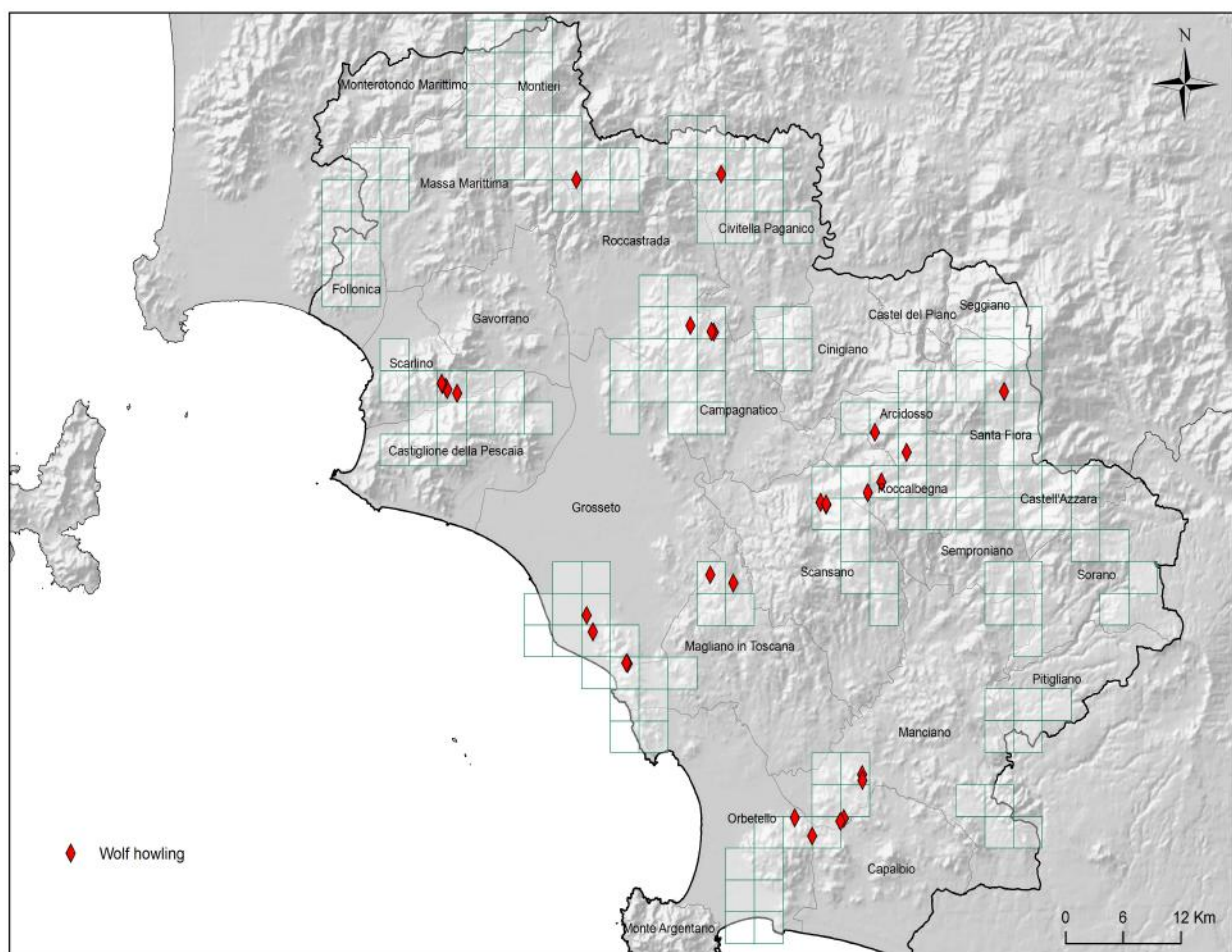


Figure 8. Distribution of howling replies obtained in the project area.

Combining all data collected with the different research techniques we estimated the presence of 21 packs in the project area (fig. 9). We recorded 16 of those as reproducing packs, defined on the basis of the presence of pups registered by wolf-howling or camera trapping. For the remaining 5 we did not collect any evidence of reproduction.

In the absence of signs of reproduction we defined a pack when the following conditions were met:

- Identification of at least 2 genotypes sampled for the entire survey period, or resampled in subsequent months, in an exclusive spatial cluster.

- Presence of at least 2 non-resampled genotypes but signs of recurring presence (scats) and videos / photos of at least 2 associated animals. By recurrence we mean signs of presence recorded in the different months of sampling.

The exclusivity of the packs is defined on the basis of the following factors placed in hierarchical order of importance:

- Simultaneity of response to wolf-howling.
- Distance between the RV (the minimum distance between replies with pups recorded was 6,13 Km, based on Ciucci et al. 2018).
- Genetic cluster of signs of presence spatially exclusive.

Following these criteria we defined a hypothesis of territorial arrangement of the 21 packs, considering a radius of 5,6 Km from the centre of the territory of each pack (we chose this distance considering a mean territory size of 100 Km²). The centre of the territory was considered the location of pup's reply, the location of pups "captured" by camera trapping, or the location of recurring signs of presence (scats, and/or video photos).

We have to underline that this is only a way to show the possible distribution of the packs over the provincial territory. The only tool to obtain a reliable estimate of the territorial arrangement of the packs is the radiotelemetric monitoring of the animals.

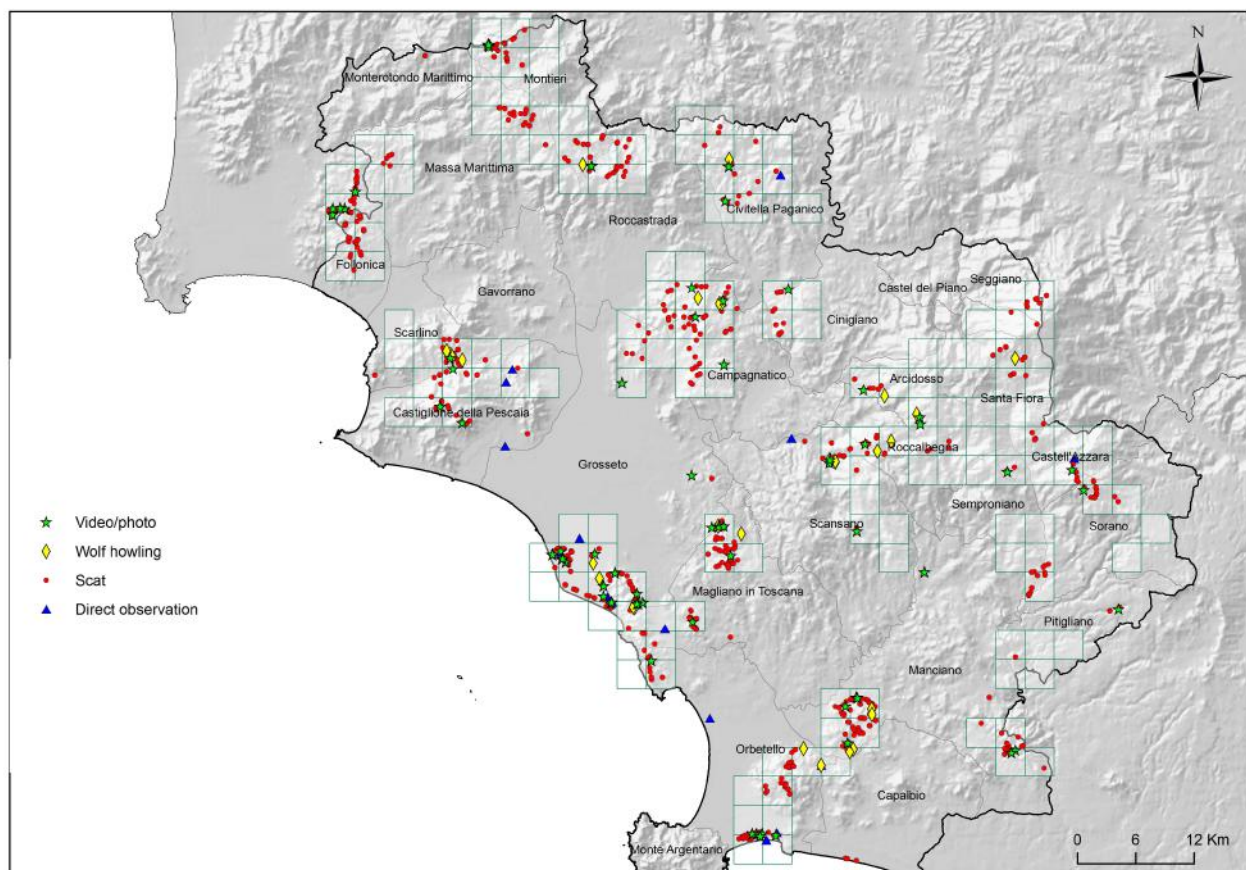


Figure 9. Wolf presence data registered in the sampling period.

4.4 Presence and distribution of the species in the Province

We have collected $n=1,836$ presence data, divided in: sightings ($N = 25$, 1%), retrieval of wolf carcasses ($N = 5$, <1%), prey remains ($N = 5$, <1%), wolf damages to livestock ($N = 6$, <1%), scats ($N = 978$, 53%), camera-trap ($N = 744$, 41%), footprints ($N = 36$, 2%), wolf howling ($N = 37$, 2%). Accounting for redundancy of presence data when multiple presences appeared with the same geographic coordinates (e.g. photos and videos of the same camera-trap), we counted them as a single presence point, thus sample size is reduced by 48%, with a final presence point data of $n = 952$.

The results of the model showed that, within the province of Grosseto, the wolf distribution range covers an area equal to $2,216.09 \text{ km}^2$ (49.47% of the project area), composed by the 55.1% of suitable areas and, consequently, by the 44.5% of unsuitable areas. Overall, suitable areas covered an area equal to $2,838.4 \text{ km}^2$ (63.4% of the province of Grosseto). However, not all suitable areas are included inside the wolf distribution range: in fact, the 70.5% of suitable areas are within the distribution range (consequently, the rest 29.8% are outside it) (fig. 10).

4.5 Estimation of population size

To make a reliable estimate of population size we applied capture-recapture models to the individual genetic capture histories.

We considered only the months from April to August 2017 for the capture-recapture analyses, in order to exclude the pups of the year from the population estimate and obtain an estimate of all individuals excluding pups. As a consequence, the number of detected individuals reduced to 51 (27 females and 24 males).

The estimated number of individuals present in sampling area, following the most reliable model, was 80 (95% CI: 50-109). The apparent survival of the individuals over 5 months (from April to August) was 0.47 (95%CI=0.07-0.87) and the average detectability was 0.34 (0.20-0.48).

In order to make an inference of population size from the sampling area ($N = 80$) to the whole province of Grosseto we used the following rationale:

- the sampling grid covered a surface of $1,417 \text{ Km}^2$
- each genotyped sample may be referred to either a resident wolf or a floater; for floater is arbitrary and possibly of little use trying to estimate the extent of area used, while for resident wolves we can approximate it to the average HR size in the Apennines (i.e., 100 km^2 , radius = 5.6 km, Mancinelli et al. 2018)

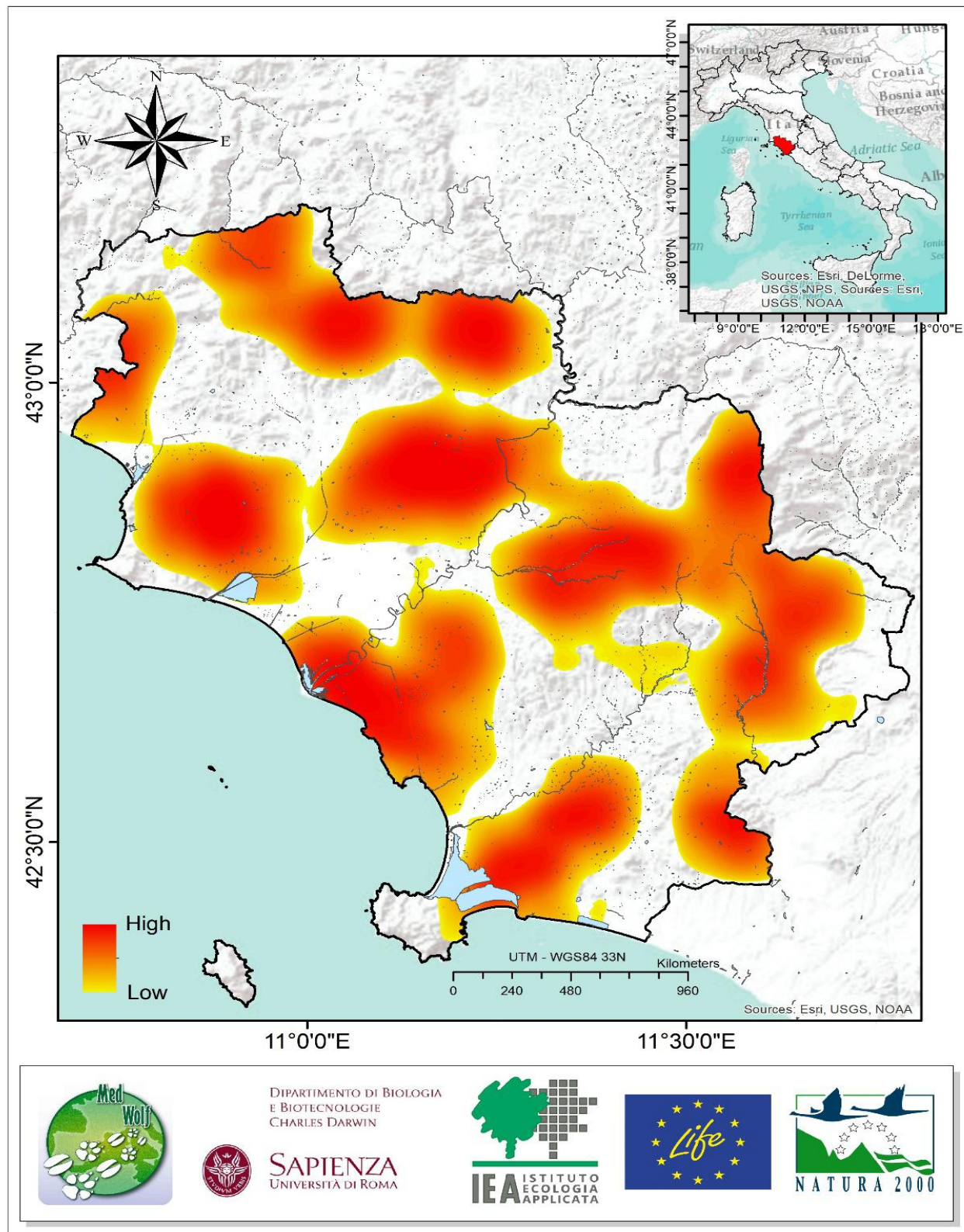


Figure 10. Distribution of the wolf in the province of Grosseto estimated by ordinary-kriging (Geospatial Analysis extensions, ArcGIS 10.2) and based on 952 signs of presence collected from May 2017 to August 2017. The distribution is represented as a continuous density map, where higher values (in red) show higher density of presence location points, and lower values (in yellow) show lower density.

- Each wolf, corresponding to each sampled genotype (assuming it is of a resident wolf), is therefore expected to possibly roam beyond the frame of the sampling grid, with samples located along the external border of the sampling grid expected from wolves on averaging wandering 5.6 off the sampling grid.

We therefore externally buffered the sampling grid by 5.6 km and assumed the resulting area (4,152 Km²) was the area where the estimated (resident + floater) wolf population resided during the sampling period. **This corresponds to 1.9 wolves/100 km²** (IF 95%: 1.2 22.6 wolves/100 Km²).

In order to estimate the number of packs in the whole provincial territory we used the following rationale:

- suitable landscape accounted for 2,216 Km² at the province scale (49.5%, total of 4,479 Km²), and 939 Km² within the sampling grid (66.2%, total of 1418 Km²)

- 139 Km² of suitable habitat occurs outside the buffered sampling grid (i.e., sampling grid + 5.6 km external buffer), conceivably hosting an additional number of wolves/packs.

- Seven continuous/cohesive areas comprising suitable habitat occur outside the buffered sampling grid, ranging in size 19.0280.0 km², for a total of 326.9 Km². However, 2 of these comprise <30% of suitable habitat, so that the total non-sampled area featuring >30% of suitable area (i.e., 197.8 Km²) would correspond to 3.8 wolves assuming the same wolf density as in the sampled area, or a total of 84 wolves comprising the (buffered) sampled area.

On a coherent line of reasoning, a minimum of 21 packs have been detected in the province (see paragraph 3.4) and they are all consistent with the kriging-estimated range. However, as the sampling did not cover the entire province area, according to the occurrence of suitable areas outside the buffered sampling grid 1 pack could be expected in the central-western non sampled area, and also 2 additional packs considering the transboundary suitable areas (N-trans and S-trans), providing for the following final estimates:

- 22 packs (21 sampled + 1 likely present but unsampled)
- 24 packs (21 sampled + 1 likely present but unsampled + 2 in common with adjacent provinces but non-sampled and excluding Argentario) (fig. 11)

Following a more empirical approach to population estimation, and by assuming each pack was composed during the sampling period by 3-4 wolves each, plus an additional 20% of floaters (Fuller et al. 2003), this would correspond to:

Packs	Resident wolves	Floaters (20%)	Total no. wolves
21	63-84	12-17	75-101
22	66-88	13-18	79-106
24	72-96	14-18	86-115

Note that the estimates above, empirically corrected for non-sampled areas (packs), fall within the statistical uncertainty of the POPAN estimated wolf population, therefore (a) lending confidence in this estimate, and (b) indicating that this estimate, including its uncertainty, can be used for the whole provincial scale. However, minimum pack number empirically established at 21 should be lifted to 22-24 according to extrapolation of size and quality of unsampled areas.

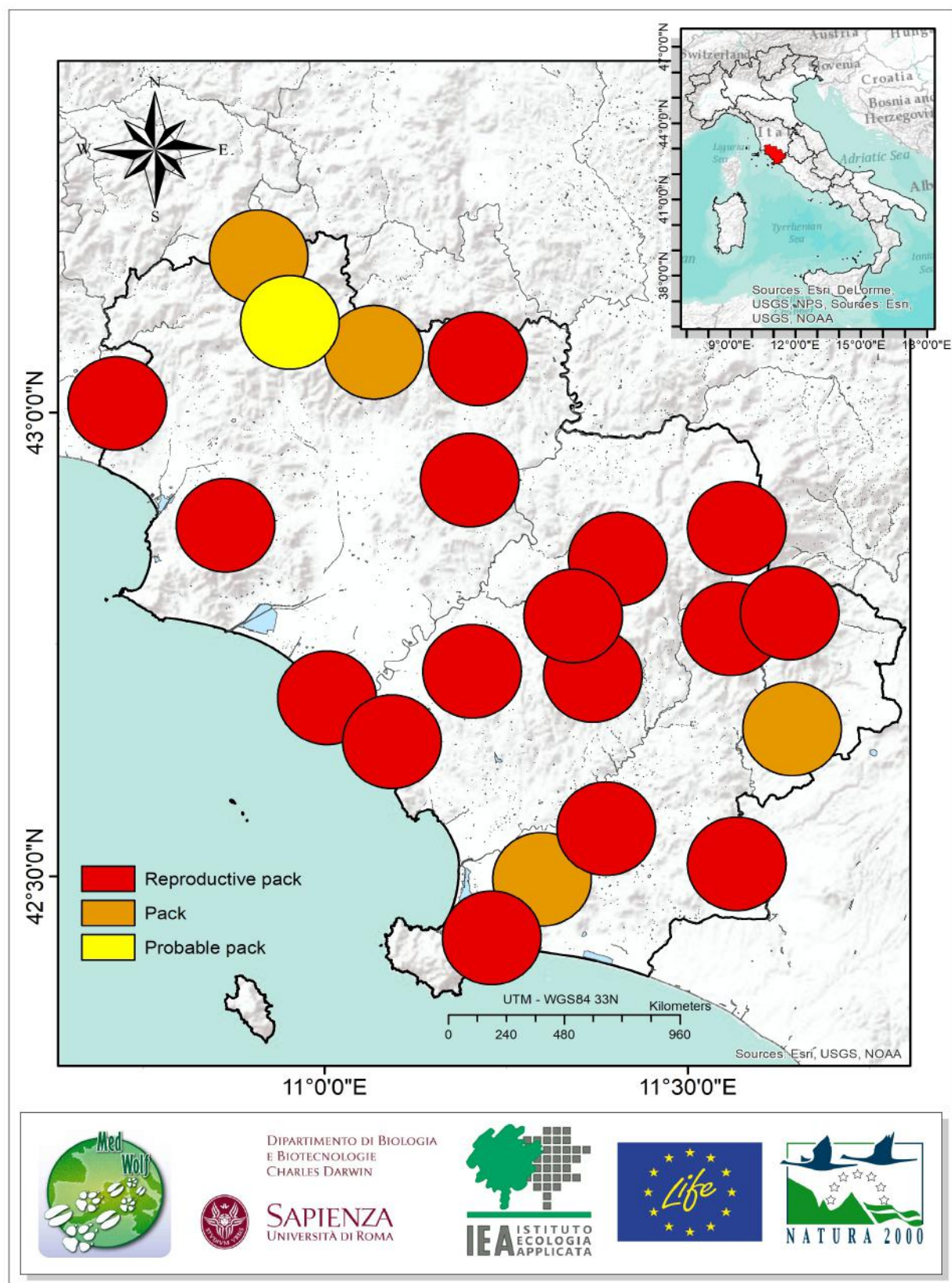


Figure 11. Hypothesis of the packs distribution in the province of Grosseto.

5. CONCLUSIONS

The action was carried out successfully and we reached all the objectives foreseen.

The methodological approach followed in this study could be a good example to be followed in the future not only in the project area, in fact, we produced a reliable and accurate estimate of wolf distribution and abundance at the provincial level and the results achieved could represent a starting point to monitor the evolution of wolf presence in the future.

The presence of the wolf was detected from the mountains to the seaside, and the results obtained seem to indicate that the species has occupied all the available territory.

The apparent survival of the individuals over 5 months (from April to August 2017) obtained from the application of mark-recapture suggests that there is a high rate of mortality or emigration from the study area, if we consider that this estimate does not include pup's mortality, and a possible high turn-over of the individuals.

6. LITERATURE CITED

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ANNEX 1

Non-invasive capture-recapture estimate of gray wolves (*Canis lupus*) in the Grosseto province, Italy

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Data Used

We performed the analyses on the data provided by the EU Life MEDWOLF (LIFE11 NAT/IT/069) project. The files used for the abundance estimate consisted of:

- 1 file containing the list of detected genotypes and date of capture (file name: DataBase+Results-MEDWOLF.xlsx)
- 3 files describing the systematic effort per day (file names: Sforzo di campionamento Lucchesi.xlsx, Sforzo di campionamento Morimando.xlsx, Sforzo di campionamento Fazzi.xlsx)

Wolf scats were collected from the 30th of March to the 28th of September, encompassing one wolf breeding/pup rearing period (April-October, Mech 1970). We pooled the 3 effort files and produced a single effort file stored in the file Medwolf_results.xlsx. A summary of the systematic monthly effort and of the collected genotypes is presented in Table 1.

Table 1. Summary of monthly systematic effort (expressed in km) and of number of wolf genotypes that were successfully detected.

Month	Effort (km)	Genotypes
April	857	20
May	13	7
June	627	32
July	10	8
August	44	8
September	669	20

- Capture Matrix

We compiled the daily capture matrix of the 63 genotyped wolves and putative wolf x dog crosses which is stored in the file named MEDWOLF_RESULTS.xlsx. A capture matrix is a binary table with individuals in rows and sampling occasions in columns. The entries of the matrix are 1s if the individual is detected in a sampling occasion and 0s if it is not detected. A total of 63 individuals (including wolves and wolf x dog crosses) were detected (32 males and 31 females). We visualized the detection of new genotypes over time by plotting a rate of discovery curve (Fig. 1). The curve reaches a plateau in mid-July, indicating that few new genotypes were sampled in the month of July and August. The slope of the curve becomes steeper in September, indicating the input of new genotypes in the sample during the last month of the study. We considered only the months from April to August for the capture-recapture analyses, in order to exclude the pups of the year from the population estimate and obtain an estimate of mature individuals. As a consequence, the number of detected individuals reduced to 51 (27 females and 24 males).

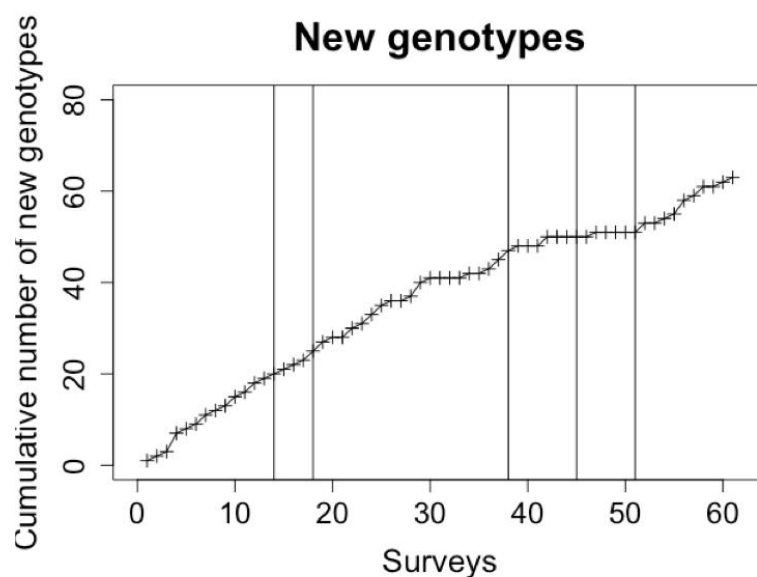


Figure 1. Rate of discovery curve of new detected genotypes (y-axis) over time (x-axis). Vertical bars represent the months in which the sampling occurred (from left to right April, May, July, August, September).

Capture-Recapture Analyses

The data were analyzed using two maximum likelihood based modelling approaches. The first is the Schwarz and Arnason parametrization of the Jolly-Seber model (hereafter POPAN estimator, Schwarz & Arnason 1996) which includes immigration and emigration dynamics in the parameter structure. The second (Capwire, Miller et al. 2005), models a close population but it is robust to closure assumption violations (Miller et al. 2005). The models were implemented using respectively the packages Rmark (Laake 2013) and Capwire (Pennel & Miller 2012) in program R (R core team 2017).

- POPAN estimator

For the analyses with the POPAN estimator the daily capture matrix was pooled in monthly capture occasions (if an individual was detected at least once during one interval it was considered detected for that occasion). The POPAN estimator has been used to estimate wildlife population abundance in several case studies in which it is necessary to take movements in and out of the population into account (e.g. Tyne et al. 2014, Gupta et al. 2017). It allows for adds and losses in the parameter structure and it provides estimates of detectability (the probability of detecting individuals, p), apparent survival (the probability that individuals stays in the study area between occasions, ϕ), entry probability (the probability that individuals enter the population, $pent$) population abundance per capture occasion (N) as well as the super-population size (N^* : all individuals that ever enter the sampled area during the study, Schwarz & Arnason 1996). We fitted a model set that included constant parameter models and models with the effect of group (sex) and time (time, effort) variables on detectability, survival and probability of entry (Table 2).

Table 2. Tested effects on models' parameters. ϕ =apparent survival, p =detectability, $pent$ =probability of entry. The + term indicates an additive effect between variables.

Parameter	Effect
ϕ	time, sex, time+sex
p	time+sex+effort
	time+effort

	time+sex
	sex+effort
	time
	sex
	effort
	time
	sex
	effort
<i>pent</i>	time

We selected the best models based on the Akaike Information Criterion corrected for small sample sizes (AICc, Burnham & Anderson 2002). The model with the lowest AICc was selected as the most parsimonious and parameters estimates were averaged if there were models within 2! AICc from the best model (Burnham & Anderson 2002).

The POPAN estimator relies on the following assumptions (Lebreton et al. 1992)

- Individuals are correctly recognized: the genetic laboratory procedures are designed to minimize errors in the genotype identification process.
- Sampling is instantaneous: the monthly capture occasions are relatively shorter compared to the whole study period (6 months).
- Capture and survival probability do not vary among individuals: we tested this assumption by running specific tests using the package R2ucare (Gimenez et al. 2017) on the whole dataset and on the female only and male only datasets. TEST2 evaluates the assumption of homogenous capture probability, TEST 3 evaluates the assumption of homogenous survival probability (Lebreton et al. 1992). The global test (TEST2+TEST3) is used as a goodness-of-fit test for the Cormack-Jolly-Seber model (Lebreton et al. 1992).

- *Capwire*

The population abundance was also estimated with the Capwire single-session estimator (Miller et al. 2005). Such model has been successfully applied to estimate population size of different species from genetic capture-recapture data (Miller et al. 2005), including gray wolves in the Northern Rocky Mountain ecosystem (Stenglein et al 2010). Capwire is a closed population estimator and models the data as if they arose from S samples of size one (the total number of captures) from the population. It assumes that all the individuals are correctly identified and all draws are independent and identically distributed. The data are therefore treated as a multinomial vector of capture counts for each individual (Miller et al 2005). The abundance estimated with this model include both transients (individuals that passed through the study area and left it) and residents. Therefore, the abundance estimate obtained with this model over the 5 months of the study is comparable with the super-population size (N^*) estimated by POPAN (all individuals that ever enter the study area during the sampling period, Kendall 1999). We performed a likelihood ratio test (Miller et al. 2005), to select between the two available Capwire formulations: the null even-capturability model (ECM) and the model accounting for 2 unequal capture probabilities (the 2 innate rates model, TIRM). We set the threshold for rejecting the ECM model as $p < 0.1$ as recommended by Miller et al. (2005). The selected model was applied on the data defined as the total number of times each individual was caught in the study (Miller et al. 2005). We fitted the model to the total sample ($S=89$ captures of 51 individuals, 1.7 captures per individual), to the female only dataset ($S=54$ captures of 27 individuals, 2 captures per individual), and to the male only dataset ($S=35$ captures of 24 individuals, 1.5 captures per individual).

RESULTS

Capture recapture analysis

- POPAN

The goodness-of-fit tests resulted non-significant ($p > 0.5$, Table 3) for all the tests, both for the whole dataset and for the female only and male only datasets showing that the collected data respect the models' assumptions of equal survival and catchability.

Table 3. Results of the goodness-of-fit tests applied on the total, female only and male only datasets. Statistic=test statistic, df=degrees of freedom.

Dataset	Test	Statistic	df	p-value
Total	TEST2+TEST3	3.31	12	0.99
Total	TEST2	3	4	0.56
Total	TEST3	0.31	3	0.95
Female only	TEST2+TEST3	3.81	10	0.96
Female only	TEST2	1.91	3	0.59
Female only	TEST3	1.89	3	0.59
Male only	TEST2+TEST3	3.41	7	0.8
Male only	TEST2	0.7	2	0.7
Male only	TEST3	2.71	3	0.44

The AICc scores of the best models (within $2\Delta\text{AIC}$) models are listed in Table 4. The best model ($\Delta\text{AIC}=0$) was the one with constant apparent survival, effort effect on detectability and time effect on the probability of entrance. Other models are supported with $\Delta\text{AIC} < 2$ (Table 4). The complete list of fitted model is stored in the MEDWOLF_RESULTS.xlsx file.

Table 4. List of the models supported by the ΔAIC . The term (.) indicates a constant parameter, (time) indicates that the parameter changes with the capture occasion, (effort) indicates that the parameter changes proportionally to the sampling effort.

model	npar	AICc	ΔAIC	Deviance
Phi(.)p(effort)pent(time)N(.)	8	167.33	0	-45.89976
Phi(.)p(time)pent(.)N(.)	8	167.66	0.33	-45.57473
Phi(.)p(effort)pent(.)N(.)	5	168.37	1.033957	-37.55354

Model averaged estimates of apparent survival, detectability and probability of entry are reported in Table 5. As shown by the best selected model (Table 4), capture probability is correlated to sampling effort (Table 5). Estimates of population size (N) per occasion (month) are listed in Table 6. The average number of wolves in the sampled area during each month is therefore 50 individuals, (SE 18, 95% CI 15-84, Table 6). This quantity is different from the super-population size (N^*), which is represented in Fig. 2 and it is estimated to be 80 (SE=15, 95% CI 50-109) 41 females (95% CI 26-55) and 37 males (95% CI 23-52).

Table 5. Model averaged estimates of apparent survival (ϕ), detectability (p), probability of entry (pent) relative standard errors (SE). 95% confidence intervals are reported in brackets.

	Effort	p	SE	ϕ	SE	pent	SE
April	857	0.50 (0.11-0.90)	0.27	0.83 (0.51-0.96)	0.11	-	-

May	13	0.14 (0.06-0.30)	0.06	0.83 (0.51-0.96)	0.11	0.13 (0.01-0.79)	0.19
June	627	0.59 (0.33-0.81)	0.13	0.83 (0.51-0.96)	0.11	0.11 (0.01-0.76)	0.16
July	10	0.17 (0.07-0.35)	0.07	0.83 (0.51-0.96)	0.11	0.03 (0-0.71)	0.07
August	44	0.20 (0.07-0.35)	0.09	0.83 (0.51-0.96)	0.11	0.03 (0-0.71)	0.07

Table 6. Model averaged estimates of total abundance (N) per capture occasion (month). N females=abundance of females, N males= abundance of males, SE = standard errors. 95% confidence intervals are reported in brackets.

Occasion	N	SE	N female	SE	N males	SE
April	53 (20-109)	28	28 (12-56)	15	26 (8-52)	14
May	54 (21-86)	17	28 (11-45)	9	26 (10-42)	8
June	53 (32-74)	11	27 (17-38)	6	25 (15-35)	5
July	47 (19-74)	14	24 (10-39)	7	22 (9-36)	7
August	42 (5-78)	19	22 (3-41)	10	20 (2-38)	9
Average	50 (15-84)	18	26 (8-44)	9	24 (7-41)	9

- Capwire

The likelihood ratio test was significant (LR=22, $p=0.008$) therefore we used the TIRM formulation of the model. Total population abundance was estimated to be 102 (95% CI 88-149), composed by 44 females (95% CI 33-64) and 62 males (95% CI 42-141). The super-population sizes estimated by the two models are represented in Fig 2.

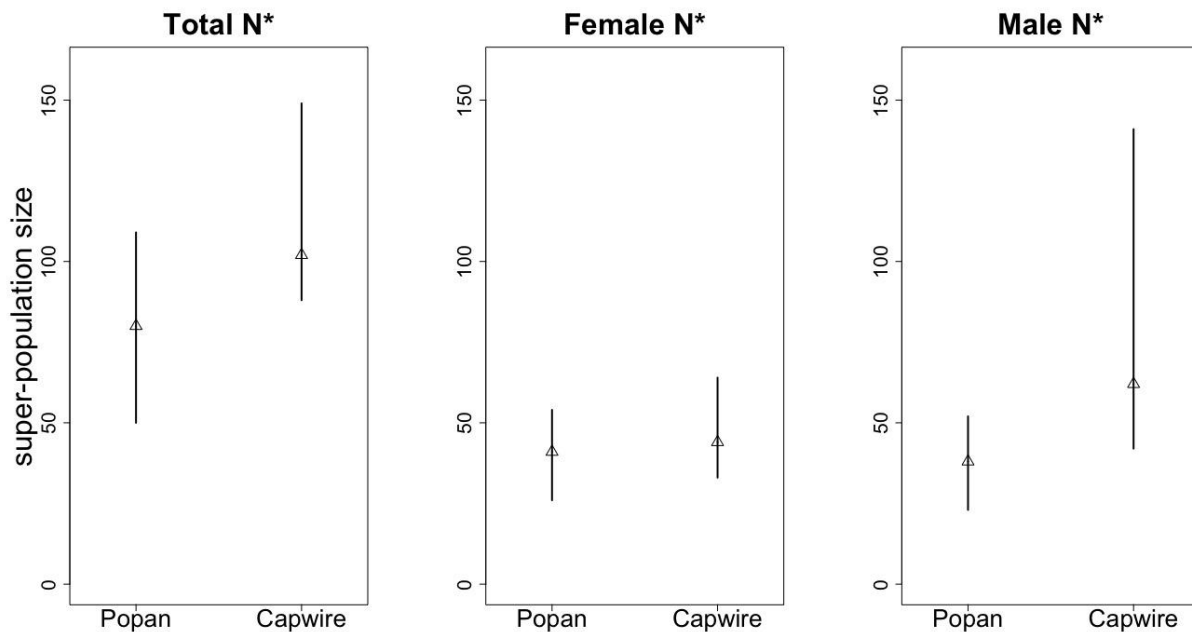


Figure 2. Population estimates obtained with POPAN and Capwire.

- Comparison between the models' results

The Capwire point total estimate is higher than the POPAN estimate by the 27% (Fig. 2, left panel). However, the confidence intervals partially overlap. A possible explanation for this difference is

that the Capwire estimator has a tendency to overestimate the real population size with small samples (< 1.7 captures per individual, Stenglein et al. 2012). Our total dataset has 1.7 captures per individual, the minimum required sample size which can explain the low Capwire estimator performance, resulting in a biased estimate. This explanation is also supported by the fact that the males only estimate (corresponding to the smaller sample size: 1.5 captures per individual) has the higher bias (63% higher than the POPAN estimator, Fig. 2, right panel), while the female only estimate has a quite smaller bias (7%, Fig. 2, central panel), relying on a higher sample size (2 captures per individual). For this reason, we recommend the POPAN estimates as the most reliable.

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