lynxconnect

Selection Protocol of Reintroduction Areas for the Iberian Lynx

Beneficiario coordinador:

Junta de Andalucía

Socios beneficiarios:

adenex, artemisan, CBD-Hábitat, Castilla-La Mancha, Castilla-La Mancha, cimbal, Estación Biológica Doñana CSIC, Diputación de Badajoz, Junta de Extremadura, Región de Murcia, Junta de Extremadura, FomeCam, FOTEX, ICNF, Infraestruturas de Portugal, WWF

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INDEX

1.Introduction

The correct selection of reintroduction areas is a key step in the process of recovery of extinct populations of threatened species (Seddon, 1999; Breitenmoser et al., 2001; Schadt et al., 2002; IUCN, 2013).

The IUCN declared the Iberian Lynx (*Lynx pardinus*) "Critically Endangered" in 2002 (IUCN 2002). The recovery process of the species has been addressed by gradually solving the most urgent problems with the support of successive LIFE projects. Thanks to the results of the conservation actions carried out since the beginning of the century, Iberian Lynx numbers have increased, and the species has been reclassified as "Endangered" (Rodríguez and Calzada 2015).

From the very beginning, the increase of population through reintroduction has been proposed as the only course of action capable of safeguarding this emblematic species in the long term (Breitenmoser et al., 2006). Releases began in 2009 and 2010 in two areas of Sierra Morena where the Iberian lynx had become extinct in the past (Guadalmellato and Guarrizas). These populations are currently consolidated, with continuous exchanges with Andújar-Cardeña population, showing a meta-population dynamic. In 2014 and 2015, reintroductions started in four areas outside Andalusia; Campo de Montiel (south of Ciudad Real, Castilla la Mancha), Montes de Toledo (Castilla la Mancha), Valle de Matachel (Extremadura) and Vale do Guadiana (southern Portugal). In 2020, the Iberian Lynx population was estimated at a minimum of 1,111 individuals. However, it is estimated that to achieve the Favourable Conservation Status (FCS), the creation of at least eight more populations (Pérez de Ayala 2019) is necessary. To achieve Iberian lynx FCS is an obligation established in the Habitats Directive (92/43/EEC) for every member state, which would allow us to consider the species safe from extinction. Therefore, it is still necessary to maintain the effort of identifying suitable areas in which to establish new Iberian Lynx populations.

The potential habitat of the species is highly fragmented (Guzmán et al., 2004; Sarmento et al., 2004). It is essential to produce a work plan that puts together a common methodological protocol for the selection of the areas of reintroduction that goes beyond political and administrative boundaries. This way, the process of recovery of the Iberian Lynx can be developed in a coherent manner from the critical initial stage in the two EU countries (Spain and Portugal) and the autonomous communities involved.

This document presents a methodological proposal for the evaluation and selection of the future areas for reintroduction of the Iberian lynx throughout its area of historical presence.

The present protocol will contribute to the development of the content of the reintroduction program of the Iberian lynx in Spain and Portugal, which is currently carried out within the Iberian Lynx Work Group. For this, a coordination mechanism for both initiatives will be established, thus satisfying a practical need as set out in the minutes of the last meeting of said Group. The integration of this protocol within the program will be ensured, taking into consideration the obligations and commitments acquired by the beneficiaries of LYNXCONNECT in relation to the fulfilment of the LIFE project.

The program will be the technical and scientific document that will be sent by the Work Group to the Spanish Sectorial Conference on the Environment for its approval, at which time it will become binding for Spain. In the case of Portugal, the document will be sent through the Multilateral Commission, to said country for formal recognition and to be validated by the Executive Commission of the “Plano de Ação para a Conservação do Lince Ibérico em Portugal – PACLIP 2015-2020”.

This protocol is an update of the protocol elaborated in the framework of the Iberlince LIFE project (LIFE10NAT/ES/000570). The updates incorporated are based on the knowledge and experience gained during the selection process of the reintroduction areas and during the reintroduction

process itself. The original version of these protocols was drafted taking into account what was established in a series of reference documents and experiences:

- The recommendations of the document "Guidelines for reintroductions" prepared by the International Union for Conservation of Nature (UICN) Reintroduction Specialist Group.
- Scientific and technical bibliography, in particular, the works of Rodríguez and Delibes (1992), Guzmán et al. (2004) and Sarmento et al. (2009) and those carried out in Portugal by the Instituto da Conservação da Natureza e Biodiversidade (ICNB) in 2009, 2010 and 2011, coordinated by P. Sarmento.
- The gained experience, the conclusions and the work carried out within the projects LIFE02 NAT/E/8609, LIFE06 NAT/E/000209, LIFE94/E/ A222/E01189/CAL, LIFE98 NAT/E/5343, LIFE NAT 02/E/8617, LIFE+ NAT 07/E/000742, LIFE B4-3200/94/767, LIFE B4-3200/99/00642, LIFE06 NA- T/P/191, LIFE08 NAT/P/227.
- The contents of the Plano de Acção para a Conservação do lince ibérico (PACLIP) (Portugal) – PACLIP 2015-2020.
- The guidelines and procedures established in the Estrategia para la Conservación del lince ibérico (Strategy for the Conservation of the Iberian Lynx) (Spain).
- The Memorando de Entendimiento sobre colaboración transfronteriza para la conservación del águila imperial y el lince ibérico (Memorandum of Understanding on Cross-Border Collaboration for the Conservation of the Imperial Eagle and the Iberian Lynx) (1 October 2004), signed by the respective Ministers of Portugal and Spain.
- The Acuerdo de Cooperación entre la República Portuguesa y el Reino de España (Cooperation Agreement between the Portuguese Republic and the Kingdom of Spain), concerning the Programa de Cría en Cautividad del lince ibérico (Iberian Lynx Captive Breeding Program) (31 August 2007 in Lisbon), which created a Joint Commission for the Conservation of the Iberian Lynx.
- The Protocolo de cesión (Transfer Protocol) of Spain to Portugal of Iberian lynx specimens (28 June 2009 in Penamacor).
- The Iberian Multilateral Commission for the Conservation of the Iberian Lynx, created by agreement between the Ministry of Agriculture, Food and Environment of Spain, the Junta de Andalucía, the Community of Castilla-La Mancha, the Junta de Extremadura and the Ministry of Environment and Regional Planning and Regional Development of Portugal.
- Reintroduction projects carried out in other countries with species similar to the Iberian Lynx, such as the reintroduction of Canadian Lynx (*Lynx canadensis*) in Colorado (Colorado Parks and Wildlife)

2. Selection process of reintroduction areas

The main goal of this protocol is to identify potential areas for long-term viable Iberian Lynx populations. The size of the reintroduction areas will be the minimum necessary to keep viable populations in the long term. In line with the National Strategy for the Conservation of the Iberian Lynx (agreed on in the Environment Sectorial Conference on 30th May 2008), the surface of the reintroduction area must be at least 10,000 ha of suitable habitat, i.e., a suitable rabbit population structure and density. This protocol also details other variables that must meet minimum requirements to be considered adequate or suitable.

The selection of reintroduction areas has two stages:

1) Large-scale areas selection: the objective is to detect, on a regional scale, the areas that maintain minimum general conditions for the settlement of the species.

2) Detailed-scale areas selection: the objective is to evaluate with as much detail as possible the pre-selected areas in the first stage of the process, through the study of the key factors that condition the ecological requirements of the species and, therefore, the actual viability of the potential population of each area. From this step, the necessary information to make the final decision on the reintroduction areas is obtained.

2.1 Large-scale areas selection

This section addresses the availability of suitable habitats. As a product, a habitat suitability model has been generated for the Iberian Lynx on a peninsular scale, represented on a map where the areas with suitable habitat conditions for the species will be identified.

The purpose of habitat suitability models is to identify suitable sites for the survival of populations of a species by identifying their environmental requirements. For the construction of the models, known data on species distribution are mathematically or statistically associated with different environmental variables. The result of the analysis indicates with some probability value (and associated statistical error) the geographic space that is suitable for a species.

The modelling is carried out using as input data the presence sites of the species and variables associated with each of these presence sites. The algorithm used translates the equation to the geographic space generating a raster layer whose values represent, on a relative scale (usually from zero to one), the environmental favourability of each species, so that high values represent those locations with better environmental conditions for the species.

In the framework of the LIFE Iberlince project, two habitat quality models for lynx were developed. In the first one, modelling was carried out using MaxEnt, which uses artificial intelligence combined with the principle of maximum entropy. This model was the basis for the map of potential habitat areas (see process below), which was applied in the selection process of the reintroduction areas created in the project. Within the same project, but as part of the necessary actions to carry out the connectivity analysis between the proposed populations, Santiago Saura and collaborators (ESIT de l UOM) elaborated another habitat quality model for the lynx. This model has not been further processed to identify potential areas for populations of the species.

The main characteristics of the above models are shown in the table below:

	Iberlince	Saura and collaborators
Method	MaxEnt	Conditional Logistic Regression
Origin digital terrain layers	Vegetation: Corine Land Cover 2006	*Vegetation:* Corine Land Cover 2006 *Roads:* BCN200 (Ministry of Development, Spain) Base cartography 1:200.000 of Serviço Geográfico do Exército (Portugal). *Slope:* MDT*25 of the National Geographic Institute (Spain) MDT to Portugal Continental e Regiões Autónomas
Presences	Stable lynx distribution for the period 2010-2012. Generated by photo-trapping, radio-tracking, latrines and geo-referenced footprints.	Locations of territorial lynx. Generated by GPS collars. Years 2008-2013.
Scale (grid dimension)	500x500m	90x90m
Variables	*Vegetation:* Artificial Surfaces Agricultural Areas Agroforestry Systems Hardwood forests Coniferous forests Mixed forest Natural pastures Moors and mesophile scrublands Sclerophyllous scrublands Transitional forest scrubland	*Vegetación:* Not suitable Non-rainfed non-tree crops Irrigated non-tree crops Olivares Other tree crops Mixed crops Mix of crops and natural vegetation Agroforestry Forest Pastures and meadows Scrub Transitional forest scrubland *Roads:* High density roads Low density roads *Slope:* Slope of the land

A MaxEnt model was processed to delimit suitable habitat patches with the necessary characteristics to support populations of the species. To this end, the following actions were carried out on the model:

1) As the finished model generates continuous favourability values, these became binary (presence-absence) by setting a threshold from which the habitat is considered suitable for the lynx. This threshold was established based on the Maximum Sensitivity and Specificity test, which is automatically calculated by MaxEnt. This threshold allows the evaluation of the potential distribution while minimising omission errors.

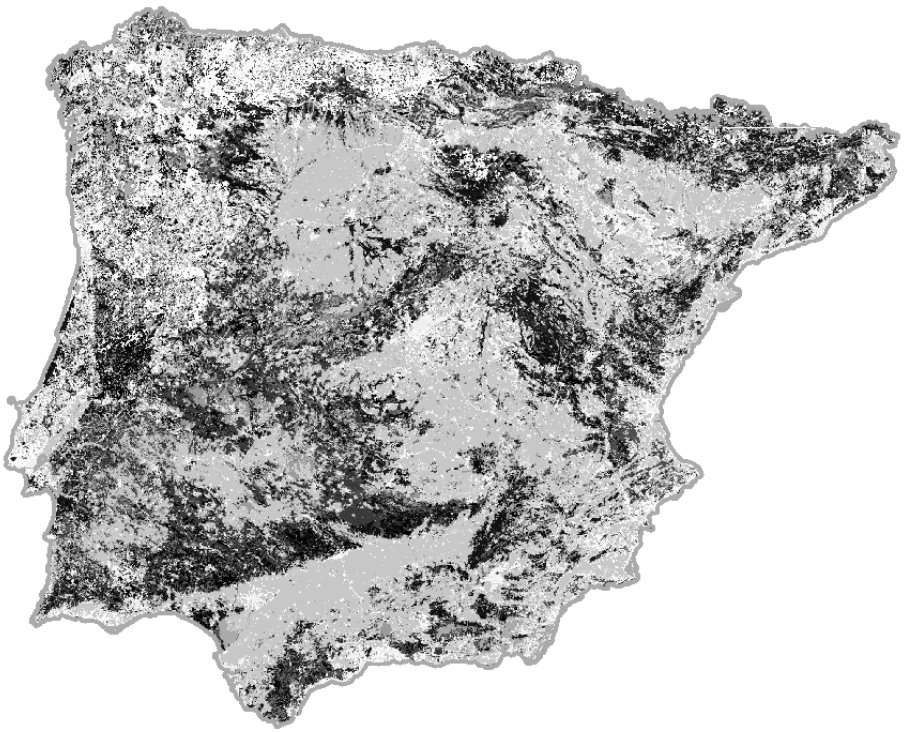
2) The altitude is a filter imposed by the availability of food. For this reason, once the model was produced, those areas over 1300 m were eliminated, threshold for the presence of the European rabbit.

3) The National Strategy for the Conservation of the Iberian Lynx (adopted by the Sectorial Conference on the Environment on 30 May 2008) establishes that the minimum surface area which could house a viable population in the long term is 10,000 continuous ha of suitable habitat. Therefore, those patches of suitable habitat below said surface area were eliminated from the model.

The resulting map was used in the selection process of reintroduction areas created in the framework of the LIFE Iberlince project. This map has also been used as a basis for conducting small-scale area selection works in projects currently underway in the Autonomous Communities of Cataluña and Comunidad Valenciana.

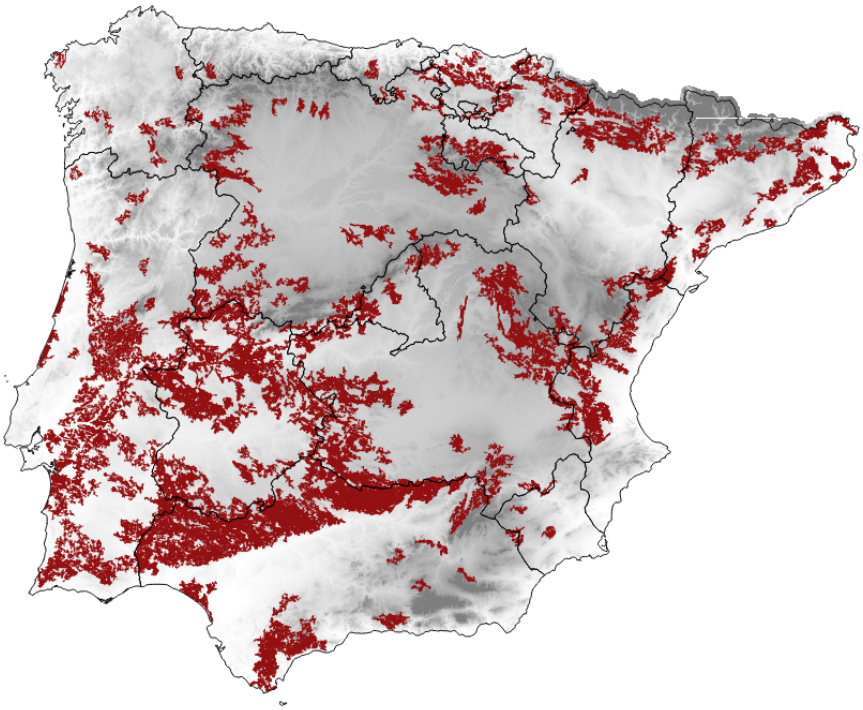
Results

The map generated by MaxEnt was the following:



The map represents a gradient of habitat quality for the Iberian Lynx. The lighter areas indicate areas of poor habitat quality, while the darker areas represent those of highest quality.

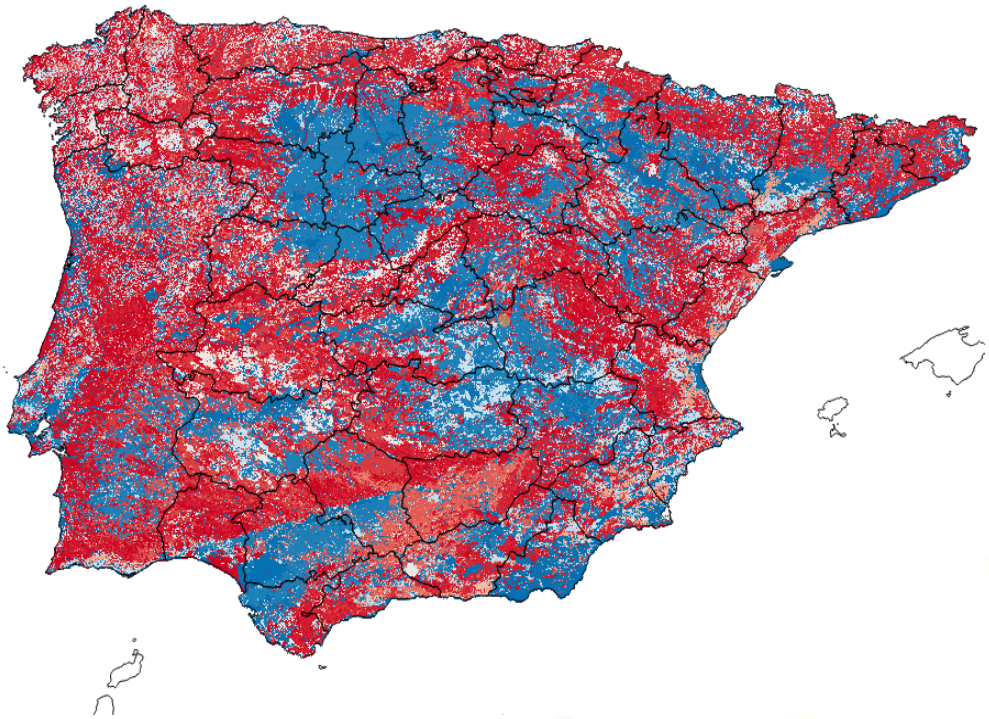
The resulting map after the application of the cut-off threshold, the elimination of suitable habitat fragments of less than 10,000 ha, and the elimination of areas above 1300 m, showing the most important areas, is as follows:



An extensive area of suitable and virtually continuous habitat is found in the south-western quadrant of the peninsula, limited to the South by the Guadalquivir Valley. All these areas would be potentially connected from the point of view of habitat. The model has identified suitable habitat areas in the northeastern quadrant of the peninsula, which are areas outside the recent historical range of the species, but that retain potential today. These areas should be considered in the future as possible reintroduction areas as long as they meet the rabbit abundance requirements of the detailed-scale habitat selection procedure.

Variables traditionally used in these types of studies, such as the presence of highways, roads, or distance to urban centres, have not been included in this model. Highways and roads are mortality factors, but do not limit the presence of the species. It depends on the permeability, maintenance, and characteristics of the road. The same applies to the variable "distance to urban centres". In recent years, stable presence and reproduction of Iberian Lynx has been observed in semi-urbanised areas, very close to urban centres. This behaviour possibly depends on the degree of tolerance and the intensity of predator control exercised by the inhabitants of each zone. These, and other related variables, will have to be considered in the detailed-scale area selection study. Other requirements already established in LIFE Iberlince should be adapted in the context of LIFE LYNXCONNECT (defragmentation, health, and social acceptance).

This is the map generated by Saura and collaborators:



Both modelling processes have been published as scientific papers with the following references:

Garrote G, Fernández-López J, Rojas E, López G & Simón M. 2020. Planning the peninsula-wide recovery of the Iberian lynx: identification of favourable habitat areas. Mammalia, 84(5), 413-420.

Gastón A, Blázquez-Cabrera S, Garrote G, Mateo-Sánchez MC, Beier P, Simón MA, Saura S. 2016. Response to agriculture by a woodland species depends on cover type and behavioural state: insights from resident and dispersing Iberian lynx. Journal of Applied Ecology. DOI 10.1111/1365-2664.12629.

For the work developed with Maxent, there are minor changes in the methodology and resulting model between the one presented here (version applied in the selection of reintroduction areas of LIFE Iberlince) and the one published in scientific journal.

2.1 Detailed-scale areas selection

In the pre-selected large-scale areas, 2.5 x 2.5 km squares will be established as sampling units, whose surface area (625 ha) corresponds approximately to the average size of the vital domain of a breeding female. The study area will therefore be divided into UTM squares of 2.5x2.5 km in which the following 13 variables will be assessed:

- Rabbit abundance
- Habitat quality
- Assessment of social attitudes towards reintroductions
- Number of carnivores killed on asphalted roads and railway tracks
- Length of the sections of highways and railway tracks with high risk of deaths
- Number of irrigation channels and ponds
- Number of complaints ratified by the environmental authorities for trapping, poisoning, shooting, hunting or any other illegal method

- Number of exceptional authorizations
- Number of trapping events detected by active field survey
- Risk of interference from domestic animals
- Data on the health status of the fauna that lives with the lynx
- Connectivity
- Area agreed upon
- Legal protection figures

Both rabbit abundance and habitat quality variables will be considered mandatory. In other words, if the total area of squares considered "suitable" (according to values indicated in the variables description) for both variables is less than 10,000 ha, the assessed area will be discarded as a reintroduction area. The rabbit abundance variable must be measured within the year preceding the start of lynx releases.

The other variables will not be mandatory as long as the reasons for being considered " unsuitable" can be corrected.

For some variables (e.g. risk of interference or road kill rates) is difficult to establish objective thresholds above which they are considered "unsuitable" and which are equally applicable throughout the peninsular geographical range. However, technicians are expected to act with a precautionary principle in mind, as being permissive will end in the death of individuals of the species.

There is a background of reintroductions projects halted due to wrongly rating these descriptor variables. Sierra Norte de Sevilla initially met the minimum conditions of rabbit abundance and habitat quality. However, the reintroduction process was halted due to an initial social rejection of reintroductions. This aspect was worked on until a favourable environment was achieved. However, during that process, and thanks to the continuous monitoring of the established variables, it was possible to detect how the incidence of the new variant of the EHV drove the rabbit populations below the minimum levels to consider the area suitable for reintroduction. The area was finally discarded. Sierra Arana area in Granada is another example, where the release of specimens was planned to begin in 2021. However, the high road-kill rate of carnivores, the high-traffic intensity coupled with the high average road speed will continue to condition the reintroductions until these problems are resolved.

In any case, work must be done to improve these non-mandatory parameters, as the present protocols are known to accurately identify problems and are very useful tools to prevent them and reduce mortality (Garrote 2019).

Rabbit abundance

The abundance of wild rabbits plays a fundamental role in the population dynamics of the Iberian Lynx. Rabbit abundance determines such relevant aspects as the reproductive capacity of the species, as well as its survival rates (Monterroso et al 2016; Perez de Ayala 2017). It is, therefore, essential to know the abundance of rabbits in the area to be assessed.

To carry out a rabbit density assessment by applying a common methodology to obtain comparable relative abundance indexes between areas, with a logistically feasible effort given the geographical scale to be addressed, is proposed. The proposed method is the fixed transect latrine count method. This methodology has been applied to estimate rabbit abundances in Iberian Lynx studies and conservation projects since the beginning of the century (Guzman et al 2004; Sarmento et al 2012). This has made it possible to define abundance index thresholds that affect the demography

of the species. Monitoring of the species in Andújar-Cardeña population showed that the rabbit abundance threshold for female Iberian Lynx to breed is around 10 latrines/km.

Based on a UTM grid of 2.5x2.5 km squares divided into 4 quadrants of 1.25x1.25 km, we propose a linear transect of a minimum of 750 m in each of the quadrants, with a minimum effort of 3 km of linear walking in each square, in which rabbit latrines will be counted within 2 m on each side of the transect line. Sampling shall preferably be carried out from June to August, after the period of highest rabbit population density (May to June). The period of maximum rabbit density coincides with the maximum development of herbaceous vegetation, which significantly reduces the detection of latrines, which can lead to an underestimation of the real abundance of the species. Due to the permanence of the latrines from the period of maximum abundance and during the summer period, as well as the maximum detectability of the latrines when the grass dries out (June-July), the months of June-August are suggested for the elaboration of the transects. The transects will be recorded with a GPS device and all latrines will be geo-referenced.

For each square, a Rabbit Kilometric Abundance Index (KAI square) will be obtained:

KAI square = total nº of latrines/total kilometres covered

Calculation of reference abundance for an area:

Only squares with KAI values greater than 10 latrines/km will be considered suitable. Only suitable squares will be considered when estimating the total suitable area, as well as for estimating the average reference abundance of rabbits in the area. This abundance will be expressed as the **median** of the abundance values obtained in those squares that exceed the threshold of 10 latrines/km. The use of the median rather than the mean has been chosen because of the contagious and irregular distribution of rabbits, which can lead to the existence of very high-density squares that may have a significant effect on the value of the mean of all squares, giving a false idea of a high rabbit density in the whole area. Using the median eliminates the effects of extreme values and gives a more realistic picture of rabbit distribution in the study area. There is a consensus that the minimum threshold for considering an area as suitable for the rabbit abundance variable is a median value of 20 latrines/km over an area of 10,000 ha, considering only those squares with KAI values over 10 latrines/km.

The methodology for estimating rabbit abundance is described in detail in the "Protocol for monitoring wild rabbit populations".

Rabbit abundance throughout the study area should be assessed within the year preceding the beginning of releases. Given the variability often found in rabbit population trends, it would be advisable to have multi-year abundance series for the species in the area.

Habitat Quality

A walking distance of at least 4 km will be carried out in each 2.5x2.5 km UTM squares (this may vary depending on the heterogeneity of the square). The variables are estimated visually in circular areas of 25 m in diameter located every 500 m. The itinerary should be designed to maximise the registration of habitat variability in each the square. To optimise time and resources, this sampling can be overlapped with rabbit latrines censuses.

Previous studies identified that the average scrub cover in territorial lynx territories was 55% (Palomares 2001). Similar values were obtained for territorial specimens from Vale do Guadiana (average scrubland = 61% min = 20 max = 91; SE = 14.89). We will consider an upper and lower range of 25% around this average value as the range of suitable habitat for the settlement of the species; a square with scrubland cover between 20% and 80% will be considered suitable. The total suitable area for the scrubland cover variable shall be the area resulting from the sum of the areas of the resulting suitable squares.

The estimation of these variables is subject to various methodological problems, such as the high inter-individual variability in estimating the scrubland cover, or the difficulty in designing sufficiently representative itineraries of the real habitat quality of the square. The reliability of using digital layers to estimate the habitat quality of each assessed square is being evaluated, which would allow an objective estimation of this parameter. Therefore, this aspect will be reflected in the **Pending Issues** section of this protocol and will be incorporated in future revisions.

Assessment of social attitudes towards reintroductions

Following IUCN's recommendations, for final decision making an objective assessment of social attitudes in selected areas should be carried out to minimise the chances of reintroduction failure.

The evaluation should have the following objectives:

a) To study the attitudes in society in the territories towards the lynx and other carnivores, and what changes have occurred.

b) To analyse local attitudes towards the Iberian Lynx, studying the locals' knowledge of its biology and the level of tolerance related with its reintroduction in each pre-selected area.

c) To assess the attitude of key members of society towards reintroduction projects with the aim of showing reasons behind those different opinions in order for us to gain a better understanding of possible negative views.

d) To elaborate recommendations to managers responsible for the reintroduction process, considering the decision-making levels and the relationship with the local level.

Surveys should be made in each area, targeting key members of society who have authority to make decisions on lynx territories (planning, forest management, agriculture, hunting, local management) or with interests in lynx territory usage (e.g. tourism, leisure, research, artistic interest, etc.).

The profiles used in previous assessments were of landowners, farmers, mayors, game managers, gamekeepers, hunters, tour operators and those involved in nature activities, as well as the general public. During the design and planning of the study, profiles and potential interviewees in each area should be identified.

Evaluations should aim at achieving the following results:

- Contextualisation of each geographical study area
- Assessment of the public knowledge of the lynx and lynx biology in the area
- Public attitudes towards reintroduction
- Opinions and their reasons
- Tolerance to the presence (coexistence) and possible attacks on domestic animals
- Conclusions and Recommendations: characterise potential opposition or support for the project in quantitative and qualitative terms

We consider the convenience of establishing a standard survey, with a minimum of objective and common questions for all the evaluated zones, for the results to be quantifiable and comparable. The elaboration of this common proposal remains a pending issue to be included in the next revision of the protocol.

Number of road-kill carnivorous in paved roads and railways

This fauna group is used as a model to assess the risk of being run over and to detect black spots for the Iberian Lynx. Ideally, all sections of asphalted roads and railways within the selected area should be

sampled. If complete sampling is not possible, efforts will be focused on those sections of tracks passing through scrubland or forested areas.

We propose to carry out transects in order to count carnivores that have been run over (lynx, fox, genet, mongoose, wildcat, otter and badger), following these specifications: A total of 12 samplings will be carried out over a minimum of six months (two samplings per month), ideally through the whole year (one sampling per month). Animals that have been run over will be removed from the roadway. The transects will be on foot or by vehicle at low speed (15 – 20 Km/h) to be able to detect animals on the roadway and roadsides. In the case of vehicle transects, these will be carried out by two people, the driver and the observer, in order to maintain attention and avoid unsafe situations.

The resulting rate for animals killed on the road (detected/km of road) will not be comparable between areas, as variables such as the type of habitat surrounding the roads, or differences in the abundance of carnivore populations in the area may change the values for this rate. A low road-kill rate in an area where carnivore populations are low (e.g. due to intense predator control) may lead to the erroneous conclusion that the risk is low. Road kills detected by this method will serve as a guideline to point at potentially dangerous areas and implement subsequent reduction measures.

The methodology is described in detail in Appendix 3 of the "Protocol for defragmentation of habitat around roadways and connectivity ".

Length of highways and railway sections with high risk of run overs

Roads passing through Mediterranean scrubland have been described as high probability Iberian lynx roadkill areas (Garrote et al 2018). To correct potential future lynx black spots, the road segments crossing through scrub habitats in the study area should be identified.

This is a complementary variable of the previous one that does not necessarily imply the detection of run over individuals. It is defined as the length of sections of these communication routes that cross areas of scrubland with high density of rabbits. Therefore, the information it provides may serve to direct the sampling of the previous variable.

Number of irrigation channels and ponds

Irrigation ponds or the accumulation of agricultural by-products (such as alpechín) have proven to be a threat to the Iberian Lynx. In most cases, drownings in irrigation ponds were due to poor perimeter-fencing that allowed the animals to access the pond. Once inside, as there was no way for the animal to climb out due to the slipperiness of the walls, the animal drowned.

An inventory of ponds and open irrigation channels in the study area is proposed, in order to ensure that the perimeter fencing is in good condition. The implementation of measures inside the pond are also recommended to enable the animals to free themselves if trapped.

Number of complaints ratified by the environmental authorities for trapping, poisoning, shooting, hunting or any other illegal method

In order to define what an illegal trapping art is, state and regional regulations and guidelines will be taken into account. Data from the last five years will be collected. This variable will be standardized per year and surface area. Due to the high variability in the complaint effort, it is a purely indicative variable.

Number of exceptional authorizations

Number of exceptional authorizations granted for the use of approved predator control methods in the last five years. This variable will be standardized per year and surface area.

No reintroduction areas may be selected where the minimums established by the Technical Guidelines for the capture of predator hunting species approved by the Sectorial Conference on the Environment on 13 July 2011 for Spain and Portugal Decree-Law No. 140/99, with the wording given by Decree-Law No. 49/2005 which transposes Directive 92/43/EC, are not met.

Number of trapping events detected by active field survey

2.5x2.5 km squares will be sampled for illegal methods that could compromise the survival of the Iberian Lynx. On-foot transects of at least 2 hours/4 km will be carry out, focusing on those areas within each square where the presence of illegal methods is most likely, giving priority to inhabited zones and their surroundings, areas with chicken coops, vegetable gardens, etc.

Prohibited hunting methods discovered during the sampling will be actively identified and positive findings will be mapped. Each transect, as well as those events detected, must be recorded by GPS. The information collected will be used to produce an index of the presence of illegal hunting activities and will detail the number of threats or cases/kilometre.

This information will make it possible to establish the level of mortality risk created by illegal methods in the area and to establish the areas of highest incidence, detailing where both search and prevention actions should be intensified.

Ideally, all squares in the study area should be sampled for illegal methods. If this is not possible, they should be sampled:

- All squares with reports of illegal methods during the last five years
- All squares with frequent human activities and adjacent squares to villages, residential areas, recreational areas, vegetable gardens, etc.

In total, and as a minimum, 50% of the squares in the study area must be sampled.

Risk of interference with domestic animals

Iberian lynx attacks on domestic animal species have been recorded continuously since the beginning of the 21st century (Garrote et al 2013). These attacks have been detected in all areas where the species occurs, whether in historical populations or in those recently created through reintroduction. As with other felines worldwide, lynx interference with domestic animals can lead to the death of the former as revenge by farmers or owners. This has even prevented or limited the settlement of the species in areas with a high interference rate, as in some areas of the Guadalmellato population (Garrote 2019). Therefore, it is essential to have as much accurate information as possible on the potential risk of interference, knowing in advance the locations of commercial and non-commercial livestock farms, mainly sheep, goat and poultry farms or chicken coops. Depending on the risk, it may be necessary to establish a programme for the prevention of lynx attacks on domestic animals, as well as for the payment of damages.

The methodology concerning the measurement of this variable will be addressed in a specific protocol derived from Life LYNXCONNECT action A.2.

Data on the health status of the fauna that lives with the lynx

One of the parameters to be considered in the selection process of reintroduction areas, based on

IUCN recommendations for reintroductions, is an assessment of the pathogen pressure for the species to be reintroduced. In small populations, such as those generated by Iberian lynx reintroduction during the first years of release, stochastic factors, such as pathologies, may become a major regulating factor. In addition, as areas with greater resource availability than those of current distribution are to be selected, it is expected that the density of specimens generated in the reintroduction areas will be high, therefore with a greater risk of disease. It is, therefore, necessary to assess the pathogen pressure in the areas selected for the reintroduction of the Iberian lynx and to compare this pressure with that existing in the current populations. Among the agents potentially pathogenic to the Iberian lynx, the prevalence of the three main agents that cause mortality in the species will be assessed for their potential as population regulating factors: (1) feline leukaemia virus (FeLV), (2) tuberculosis (TB) and (3) canine distemper virus (CDV). FeLV will be analysed on cat samples (*Felis catus* y *Felis sylvestris*), CDV on samples from all carnivores, and TB on samples from wild ungulates. In each area, sampling will be carried out, achieving the following numbers: 40 cats, 30 non-cat carnivores and 50 wild ungulates (samples will be distributed specifically depending on the abundance of each species in the area to be sampled). These three agents will be analysed with PCR, and TB will be analysed with a specific Ziëhl Nielsen staining and culture when necessary.

Connectivity

To achieve a large Iberian metapopulation of the species and its long-term conservation, it is necessary to encourage the existence of spontaneous exchange (demographic and genetic) between the different populations of Iberian lynx, so that these populations, and the species, can maintain themselves in a self-sufficient way in the Iberian Peninsula. It requires ensuring adequate distribution of reintroduction areas (among them and to the current stable populations). It is, therefore, necessary to analyse the ecological connectivity of the proposed new areas.

In the framework of the LIFE Iberlince project, a connectivity model was developed for the populations created during that project (Saura et al 2014). To this end, a territorial permeability model was developed to accommodate Iberian lynx dispersive movements throughout the Peninsula. This model was generated based on movement data of specimens tracked by GPS collars. From this permeability model, a resistance layer was generated, which is the basis for connectivity analysis between populations located anywhere in the Iberian Peninsula. To analyse the connectivity of potential areas with the rest of the existing populations through the application of methodological developments of least-cost path or circuit-based analysis applied to the study of ecological connectivity, is proposed. These analyses will be carried out based on the resistance layer developed by Saura et al.

Area with cooperation agreements

In order to achieve a good development of the reintroductions, it is established that, in the areas selected for this purpose, at least a minimum area of 5,000 ha and 50% of the surface of the selected area should have a cooperation agreement with the project. It must be taken into account that the agreement can be established a posteriori with respect to the moment of determining an area as selected.

Legal protection figures

To identify a territory as a reintroduction area, the fact of being part of Natura 2000 Network and Protected Natural Spaces will be considered. In addition, the existence of Management Plans of said Network and the Plans for Management of Natural Resources and Master Plans of Use and Management of Spaces will be positively valued.

Regarding to the inclusion in Natura 2000 Network, an area outside the Network can be considered territory of reintroduction if the rest of the variables that characterize it so advise, provided that the competent authority undertakes to designate it as an SCI (Site of Community Importance) within the duration of the project as required by the LIFE provisions.

3. Estimate of the load capacity

According to the National Strategy for the conservation of the Iberian Lynx (approved by the Sectorial Conference for the Environment on May 30th, 2008), a reintroduction area must be a minimum continuous area of 10,000 ha, with suitable rabbit and habitat conditions, with the carrying capacity necessary to support a long-term stable population. This criterion is validated in this protocol. However, estimating the minimum carrying-capacity in this way is considered to be imprecise and based on outdated data, and methodology to accurately value the carrying capacity for the Iberian Lynx of a given area is considered necessary. This should be treated as a priority and will be included in the **Pending issues** section**.**

4. Protocol review

This protocol will be reviewed and updated every 4 years. The next review of this protocol will take place in 2025.

5. Pending issues

This section lists the identified needs for improvement of some aspect of the protocol not addressed in this update. It is recommended that they are incorporated in the next update of the protocol. The pending issues identified are:

- Identification of the methodology for estimating the load capacity of an assessed area
- Reliability of using digital layers to estimate the habitat quality of each square
- Estimation of the dispersion distance, to assess the effective probability of connection between populations
- Development of a common structured survey for the evaluation of social perception of reintroductions.

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