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ORIGINAL PAPER

Status survey of the critically endangered Iberian lynx *Lynx pardinus* in Portugal

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Keywords Camera trapping · Conservation · Rabbit · Scat DNA analysis

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Introduction

The Iberian lynx *Lynx pardinus* is a critically endangered cat species (IUCN 2007) with a highly restricted geographic distribution, which is limited to the Iberian Peninsula. Presently, it is the most endangered carnivore in Europe (Delibes et al. 2000; IUCN 2007) and the most endangered feline in the world (Nowell and Jackson 1996). The ultimate cause of the Iberian lynx decline combines persecution, habitat loss, and decrease of wild rabbit *Oryctolagus cuniculus* densities (Delibes et al. 2000; Rodríguez and Delibes 2002, 2004; Ferrer and Negro 2004).

In Portugal, a survey based on interviews and questionnaires was used to evaluate the presence of the Iberian lynx between 1994 and 1997 (Ceia et al. 1998). The latter study identified five lynx populations (corresponding to five well-defined areas) of 40 to 53 individuals, which spread throughout 2,400 km^2. Three out of the five identified regions (Serra da Malcata, Serra de São Mamede, and

Guadiana valley) represented western extensions of Spanish populations. The most important lynx region (Algarve–Odemira) had an estimated population of 18 to 24 adult individuals (2.5–6.4 lynxes/100 km^2) and was located in the southern end of the country (Castro and Palma 1996; Ceia et al. 1998; Palma et al. 1999).

Despite the report of reproductive groups and consequent viability of the Algarve–Odemira nucleus (Ceia et al. 1998), both contemporary and subsequent research were unable to detect any lynxes. In fact, all methodologies (camera trapping, DNA analysis of potential scats, and snow tracking, when possible) failed to detect the presence of lynxes (Pinto 2000; Sarmento et al. 2001), indicating an extremely critical scenario for lynx populations.

In late 2001, the Portuguese governmental organization responsible for nature conservation (Institute of Nature Conservation [ICN] 2002) attempted to prevent the species' extinction by establishing several emergency measures. These priority actions consisted in evaluating the remnant metapopulations and defining areas for potential capture of individuals to be included in the captive breeding program and genetic reserve.

Therefore, a nationwide operation was initiated in 2002, which aimed at (1) obtaining baseline data on lynx status and distribution and (2) defining areas for the potential capture of founder individuals to commence the captive breeding program. The present study focuses on the results obtained during the 2-year field survey on the status of the Iberian lynx in Portugal.

Materials and methods

Even though the Iberian lynx's historical distribution once ranged throughout most of the Iberian Peninsula (Cabrera 1914), lynx populations have dramatically decreased in the last decade (Guzmán et al. 2005). The remarkable recent decline of lynx's distribution in Spain is emphasized (see Fig. 1) when comparing data reported by Rodríguez and Delibes (1990) to that reported more recently by Guzmán et al. (2005), which accounts for two breeding populations: (1) Doñana with an estimated population of 30–35 lynxes (three to five breeding females) and (2) Cardeña–Andújar with an estimated population of 90–120 individuals (25 breeding females).

Considering the above-mentioned inconsistency between lynx distribution in Portugal according to Ceia et al. (1998) (see Fig. 1) and other recent studies, a national survey was started in early 2002, following an agreement with the Spanish Dirección General de Conservación de la Naturaleza. This agreement allowed for the application of the same monitoring methods in all of the lynx's range (Guzmán et al. 2005). Consequently, the survey consisted in a combination of two widely used methods for carnivore population monitoring: sign searching and camera trapping (Wilson and Delahay 2001; Guzmán et al. 2005).

Surveys

The sign searching methodology was conducted using a geographic framework of 10×10 km universal transverse mercator (UTM) quadrats defined on the Portuguese lynx's historical range in accordance with previous studies (Palma 1980; Ceia et al. 1998) (Fig. 2). Other areas of potential lynx habitat located outside the previously defined range were also surveyed (Fig. 2). The fieldwork was performed between January 2002 and May 2003 (Table 1) and consisted in searching for signs of lynx presence (e.g., scats and tracks) within the UTM quadrats.

Focus zones (areas of intensive searching effort) were defined inside the quadrats according to landscape suitability (Palomares 2001). Furthermore, the searching effort in focus zones was mainly directed at those places with higher probability of detecting lynx signs, such as trails and rocky areas (higher likelihood of encountering scats) and muddy or snow-covered areas (better track definition). The UTM geographic coordinates of the locations of all signs detected were recorded using a handheld global positioning system unit.

Scats were collected using a suitable protocol for DNA analyses (Palomares et al. 1999). In the laboratory, potential lynx scats were identified according to their morphology (Robinson and Delibes 1988). Finally, scat DNA was analyzed in order to determine their specific taxonomic origin. These analyses were performed at the Doñana Biological Station (Seville, Spain), the Cáceres School of Veterinary (Cáceres, Spain), and the Laboratory of Genomic Diversity (Washington, USA).

Camera trapping is widely used in the study of cryptic and elusive carnivore species (Cutler and Swann 1999; Wilson and Delahay 2001; Moruzzi et al. 2002). Furthermore, it has been successfully used in Spain, both in the Iberian lynx national census and in other carnivore distribution studies (e.g., González-Esteban et al. 2004; Guzmán et al. 2005; Barea-Azcón et al. 2007).

Sampling areas for camera trapping were defined according to several parameters: (1) rabbit density compatible with lynx presence (Palomares 2001), (2) suitable habitat (Palomares et al. 1999), (3) proximity to known lynx populations (Cardeña–Andujár and Doñana), and (4) location of previously collected potential lynx scats (pending genetic confirmation). Consequently, the camera trapping survey area was confined to Serra da Malcata Natural Reserve, North Guadiana (Contenda–Barrancos), and South Guadiana (Mértola) (see Fig. 1). This methodology was applied between January 2002 and November 2003 (Table 2).

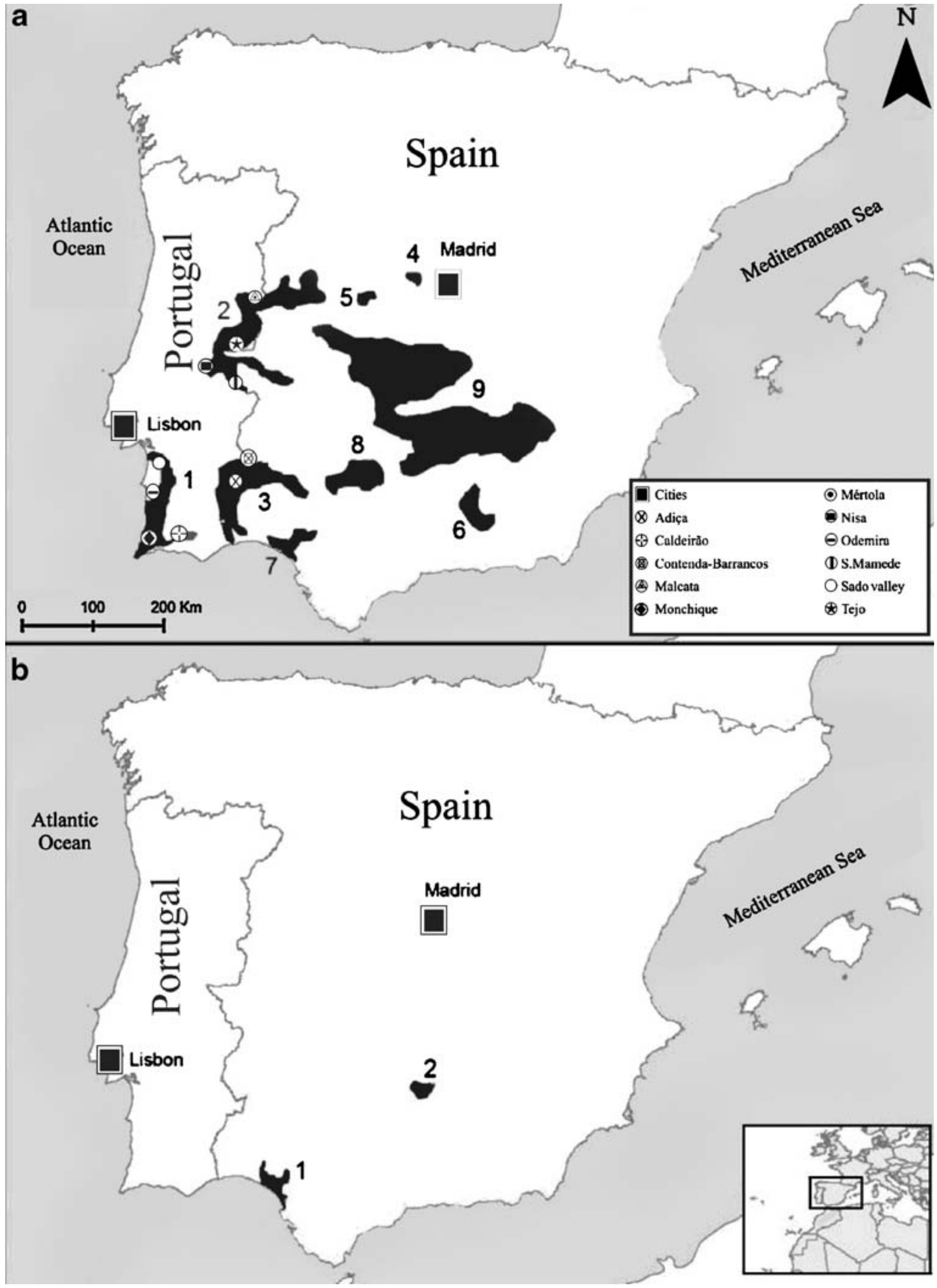

Fig. 1 **a** Geographic distribution of Iberian lynx populations according to Rodríguez and Delibes (1990) (Spain) and Ceia et al. (1998) (Portugal). *1* Algarve–Odemira–Sado valley, *2* Gata–Malcata–San Pedro–S. Mamede, *3* Western Sierra Morena–Guadiana, *4* Alberche, *5* Gredos, *6* Subbéticas, *7* Doñana, *8* Central Sierra Morena, *9* Central population. **b** Geographic distribution of Iberian lynx in Spain according to Guzmán et al. (2005). *1* Doñana, *2* Cardeña–Andujár

Cameras (pressure plate triggering devices and sensor heat activated CamTracker®) were placed on trails and trail intersections at 0.3 to 0.7 km intervals (Guzmán et al. 2005) and left for a minimum period of 28 days (Zielinsky et al. 1995).

A scent station was set up in the vicinity of each camera in order to attract lynxes into the area under direct surveillance. Scent stations consisted of a piece of cork tree bark sprayed with Iberian lynx urine (Guzmán et al. 2005). The tree bark was supported by a wooden stake (placing the tree bark at 40 to 50 cm above-ground).

A buffer area of 1.5 km (corresponding to half of the width of the Iberian lynx's home range, approximately) was calculated around the cameras, in order to determine the total area surveyed by that set of photographic traps (Karanth and Nichols 1998). Therefore, the effectively sampled area is equivalent to the polygon enclosed by camera trap locations on the perimeter of the defined buffer area (Ferreras 2001).

Results

A total of 133 UTM quadrats were surveyed (Fig. 2) with a sampling effort of 1,975.79 man-hours, corresponding to 4,252.17 linear kilometers (Table 1). A total of 313 samples

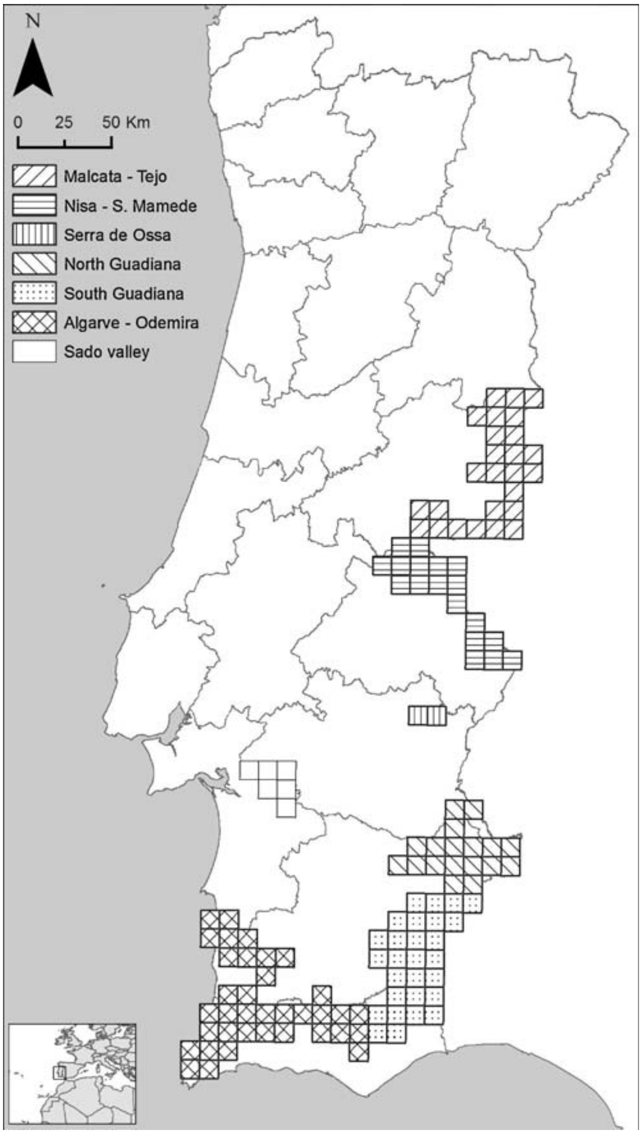

Fig. 2 UTM quadrats (10×10 km) surveyed for lynx presence during the current study

(potential lynx scats) were collected (Table 2). Roughly half of all collected scats (54%) were examined for species identity using genetic analysis.

Out of the seven surveyed areas, the highest number of sampled quadrats, highest medium linear kilometer per quadrat, and highest total effort employed in the field survey were registered in Malcata–Tejo, South Guadiana, and Algarve–Odemira areas (see Table 1), corresponding to those areas which presented the highest number of probable lynx scats (Table 2). In particular, the highest medium covered linear kilometer per quadrat (43.10 km) and highest medium effort per quadrat (17.24 man-hour) were recorded in the Algarve–Odemira area.

No probable lynx scats were detected in Serra de Ossa and Sado valley, thus precluding the use of camera traps in those areas (Table 2). The highest number of probable lynx scats was detected in South Guadiana. In fact, this area presented a much higher mean number of scats per surveyed quadrat (2.67±1.34 SE) in comparison to the other areas where probable lynx scats were detected (0.22±0.10 SE to 1.06±1.34 SE). Therefore, the highest number of camera stations (86) and camera-days (3,424) were recorded in South Guadiana.

Although 168 probable lynx scats (35% to 70% of the collected scats according to the surveyed area) were examined for species identity using genetic analysis, no DNA amplifications for lynx were registered. Additionally, all camera traps failed to detect the presence of lynx (see Table 2).

Discussion

Past and current situation

Gata–Malcata–São Pedro–São Mamede metapopulation

The 1998 census (Ceia et al. 1998) identified the area comprising Malcata, Nisa, and São Mamede (Central Western Mountains) as highly important for lynx conservation with an estimated Portuguese subpopulation of seven to nine animals, which occupied 450 km^2 approximately (medium density of 1.8 lynxes/100 km^2) (Fig. 1a, population 2). According to Delibes et al. (2000), this subpopulation was integrated in the Gata–Malcata–San Pedro–São Mamede metapopulation comprising 75–95 individuals (assuming a mean density of one animal per 21–27 km^2) (Fig. 1a, population 2) distributed over 2,050 km^2 of Iberian lynx habitat.

However, several studies using distinct methods for lynx detection, such as camera trapping and scat DNA analysis, were unable to detect lynx presence in this area since 1997 (Sarmento and Cruz 1998; Eira 1999; Sarmento et al. 2001). Furthermore, the most recent data referring to Spain describe a possible extinction of the historical lynx nuclei of Sierra da Gata and Hurdes (Guzmán et al. 2005; Alda et al. 2008) (Fig. 1b). This area was intensively surveyed during the present study and no signs of lynx presence were ever detected, suggesting an extremely negative scenario for this historical lynx nuclei.

On average, prey resources (i.e., rabbit availability) were very low in this area (Sarmento et al. 2004) and it is possible that the scarcity of prey had led to the enlargement of territory boundaries and even to home range instability. The rapid rabbit decrease and habitat deterioration in an area with already unstable home ranges and low lynx density may have prevented individuals from maintaining their territories and reproduction could have become

Table 1 Field survey parameters according to each probable lynx nuclei

	Number of quadrats	Estimated proportion of sampled quadrat (%)	Medium sampled sections per quadrat (km)	Total sampled sections (km)	Medium effort/quadrat (man-hour)	Total effort (man-hour)
Malcata–Tejo	26	17.2	20.56	352.51	7.79	257.65
Nisa–S. Mamede	18	25.8	11.06	122.06	4.94	170.29
Serra de Ossa	2	34.4	19.66	39.32	5.76	11.02
North Guadiana	17	35.29	16.43	533.80	8.56	215.52
South Guadiana	28	49.85	25.21	1695.54	9.37	678.31
Algarve–Odemira	36	68.8	43.10	1465.54	17.24	586.61
Sado valley	6	77.4	7.23	43.40	9.31	56.39

compromised (Palomares 2001). The local extinction could explain the absence of lynx data in this area. There is no reliable evidence of the presence of this metapopulation for the last 9 years (Table 3).

Guadiana valley

Ceia et al. (1998) reported an estimated population of four to seven lynxes in the Guadiana valley, occupying a range of 270 km^2 (based on an average of one cat per 67 km^2), which was highly fragmented and included the Contenda–Barrancos nucleus (irregular presence) and the Adiça nucleus (regular presence) (Fig. 1a, population 3).

The Adiça area is contiguous to Contenda–Barrancos and presents high habitat suitability for lynx including high rabbit density, which could allow lynxes to reproduce (Alves and Ferreira 2002; Sarmento et al. 2004). Furthermore, a lynx scat was found in this area in late 2001 (Santos-Reis 2003). Despite the fact that, during the present survey, both Contenda–Barrancos and Adiça were intensively prospected and camera-trapped with no positive results, a few lynx individuals may persist in the Spanish bordering area of Rosal de la Frontera and transients could be occasionally present in Adiça.

Furthermore, one must consider the location of both Iberian lynx populations identified by Guzmán et al. (2005), the recent data indicating the presence of lynxes in western Sierra Morena (Alda et al. 2008), the lynx corridor definition (according to Palomares et al. 1999), and a lynx road-kill registered less than 40 km away from the Portuguese–Andalusian border in 2003 (Pereira and Guzmán, personal communication). It is, therefore, reasonable to assume that the areas near the Portuguese–Andalusian border (Guadiana valley) present the highest probability of supporting lynxes.

Algarve–Odemira–Sado valley

According to Ceia et al. (1998), Algarve–Odemira comprised 935 km^2 of Iberian lynx habitat bearing a population of 19 to 23 individuals divided between three subnuclei (Odemira, Monchique, and Caldeirão) (Fig. 1a, population 1). The mean density of this population was one animal per 45 km^2, representing the most important Portuguese population and corresponding to 40% of all Iberian lynx range in Portugal. Also according to Ceia et al. (1998) and Palma (1994), although lynx reproduction occurred in both regions (Algarve and Odemira), these nuclei had been isolated from other populations for the last 50 years.

Table 2 Field survey results according to each probable lynx nuclei

	Scat DNA analysis				Camera traps			
	Lynx scats (probable)	Mean scats/ quadrat±SE	Scats for DNA analysis	Lynx amplification	Area polygons (km^2)	Camera-days	Camera stations	Lynx detection
Malcata–Tejo	103	0.72±0.51	36 (35)	Negative	67.95	1,465	53	Negative
Nisa–S. Mamede	8	0.22±0.10	4 (50)	Negative				
Serra de Ossa	0							
North Guadiana	31	1.06±1.34	18 (70)	Negative	50.741	758	36	Negative
South Guadiana	136	2.67±1.34	92 (67)	Negative	75.82	3,424	86	Negative
Algarve–Odemira	35	0.51±2.01	18 (51)	Negative				
Sado valley	0							
Total	313		168 (54)					

In parentheses, percentage of total probable lynx scats in which DNA was analyzed in order to determine their specific taxonomic origin

Table 3 Comparison between past and present situations of the Iberian lynx in Portugal

	Past situation[a]		Present situation
	Individuals (n)	Occupied areas (km^2)	Lynx presence
Malcata–Tejo	7–9	450	Negative
Nisa–S. Mamede	4–6	385	Negative
Guadiana valley	4–7	270	Negative
Algarve–Monchique–Sado valley	25–31	1,275	Negative
Total	40–53	2,380	

[a] Data available in Ceia et al. (1998)

The data on lynx presence used by Ceia et al. (1998) and Palma (1994) were based on sightings reported by local inhabitants. However, fieldwork carried out in this area since the early 1990s failed to produce any replicable lynx evidence (direct proofs or lynx scats identified by DNA analysis). In fact, since 1994, a total effort of 2,865 man-hours and 2,954 camera-days was applied and no evidence was ever obtained (Castro 2003; current study). Therefore, considering that there is no evidence of lynx presence in that area (except for observational data), there is a strong possibility that past estimates (Palma 1994; Castro and Palma 1996; Ceia et al. 1998) produced an extremely optimistic and unreal scenario.

With respect to the Sado region, although this basin was considered an important lynx area in the midtwentieth century, its importance was considerably reduced in recent decades due to a degenerative process including habitat destruction and prey reduction (Ceia et al. 1998). According to Ceia et al. (1998), there were six to eight lynxes occupying 340 km^2 of suitable habitat areas in the Sado valley (Fig. 1a, population 1). However, after almost 10 years of fieldwork, no consistent evidence for lynx presence was obtained, strongly suggesting that the species is absent from the Sado region.

Global situation of the Iberian lynx in Portugal

In the past, the Iberian lynx's historical distribution spread throughout most of Portugal. Presently, this feline is on the verge of extinction (Table 3). Severe rabbit regression and habitat destruction are identified as the main causes of lynx decline in recent decades (Rodríguez and Delibes 2003).

The low amount of data on lynx presence and the low habitat adequacy in most of the lynx's historical range are indicative of the concerning situation of the Iberian lynx in Portugal. Lynx conservation requires good-quality habitats where animals can settle and breed and an adequate connectivity between these areas, since the species traditionally exhibits a metapopulation structure (Ferreras 2001; Palomares 2001).

Although lynx extinction in Portugal cannot be confirmed, a highly pessimistic scenario is now drawn. No direct evidence (i.e., captured animals, road-kills, or shot animals) have been obtained in Portugal since January 1992 (see Castro 1992), thus marking the acceleration of the species extinction process, particularly in the second half of the 1990s.

Future conservation actions

The global situation of the Iberian lynx is alarming, particularly considering that the remnant populations of Andújar–Cardeña and Doñana face substantial problems related with prey scarcity, inbreeding and diseases. Recent data obtained by camera trapping on the Doñana population revealed a total of 24–33 individuals and the Andujar estimated population amounted to 60–110 animals, including 17–21 breeding females (Guzmán et al. 2005).

The increasing awareness of all the above-mentioned difficulties led the ICN to develop a conservation action plan for the Iberian lynx in order to promote a consistent and effective approach to the conservation of the species in Portuguese territory (see Sarmento et al. 2005). Recently, the efforts directed at the conservation of this species have evolved properly and important actions have been carried out, particularly since the II International Seminar and Workshop on the Conservation of the Iberian Lynx held in Cordoba in December 2004. Ongoing work focuses on establishing a methodology for evaluating reintroduction areas and several projects deal with habitat improvement and recovery of prey populations in lynx's former range. According to the captive breeding team estimates, by the year 2010, there will be 73 lynxes in captivity and it will be possible to begin the reintroduction process from then on (Vargas 2004). In November 2007, the Portuguese and the Spanish Ministers of the Environment signed a collaboration protocol regarding the captive breeding program, which will allow Portugal to fully collaborate in this process.

Although with some uncertainty, a strategy for a long-term recovery of the former Iberian lynx populations is presently established. Nevertheless, in order to actually allow lynx populations to recover, a considerable effort involving administrations, landowners, and other organizations will be necessary.

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