



Survival and space use of a restocked Iberian rabbit population in a semi-natural enclosure

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Abstract

Restocking is widely used to recover depleted wild rabbit, *Oryctolagus cuniculus*, populations in the Iberian Peninsula. However, in general, it's costly and unsuccessful. In this work, we developed a wild rabbit restocking protocol based on recommendations from existing literature to enhance its success. We released 75 Iberian rabbits, *Oryctolagus cuniculus algirus*, in a 1.7-ha restocking park, a fenced semi-natural enclosure, with similar resources as the surrounding area, in Monchique, southern Portugal. We tracked 22 radio-collared rabbits (sex ratio 1:1) for 6 months. We released some rabbits inside warrens, and others in shrub patches. After a 72-day acclimation period, we opened the restocking park passages to allow the rabbits to disperse. We then accessed rabbit survival and space use. We found that survival rates stabilised 74 days after restocking, with an estimated probability of survival at the end of the study of 35.4%. Predation by birds of prey was the primary cause of death. Regarding space use, the restocked population took $96.364 \pm \text{SE } 12.615$ days to stabilise home ranges. After the acclimation period, the maximum distances travelled by rabbits significantly increased. However, before and after the opening of the passages, the home ranges remained within the limits of the restocking park, indicating that the surviving rabbits settled within the enclosure. Rabbits released within warrens had significantly smaller core areas, while those released in shrubs exhibited more ranging behaviour. Considering our findings, we recommend implementing several measures to improve similar rabbit restocking programs.

Keywords Dispersion · Home ranges · *Oryctolagus cuniculus algirus* · Predation · Restocking park · Wildlife management

Introduction

Translocation is widely used in wildlife management to restore natural ecosystems and communities. Given the rapid destruction of natural habitats and the rising number

of threatened species, translocation is emerging as a valuable tool in conservation efforts (Griffith et al. 1989).

Translocation involves moving organisms from one area to another through human intervention. When conducted intentionally, translocations can be done for various reasons, such as political, commercial, recreational, or conservation

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purposes. When the translocation involves moving and releasing individuals into an area with an existing population, even when densities are low, it is called reinforcement or restocking. The aim is to improve population viability by, for instance, increasing population size or genetic diversity (IUCN/SSC 2013).

Restocking is a common method used to increase the populations of European rabbit *Oryctolagus cuniculus* in Portugal and Spain, whether for conservation or hunting purposes (Calvete et al. 1997; Delibes-Mateos et al. 2008b). The main goal is to establish a self-sustaining breeding core of rabbits with abundant numbers, ensuring they can naturally repopulate the surrounding areas.

In 2019, the International Union for Conservation of Nature (IUCN) classified the European rabbit as a globally Endangered species (Villafuerte and Delibes-Mateos 2019). This is mainly due to the sharp reduction in the distribution and abundance of its Iberian populations in the last few decades, particularly the southern Iberian subspecies *Oryctolagus cuniculus algirus*. The decline is primarily attributed to outbreaks of a new variant of rabbit haemorrhagic disease virus (RHDV2) (Delibes-Mateos et al. 2014; Monterroso et al. 2016; Villafuerte and Delibes-Mateos 2019). In Portugal, this species has recently been classified as Vulnerable, as it was recognised as one of the mammal species with the greatest population decline in the last decade (Mira et al. 2023).

In the Iberian Peninsula, two distinct genetic lineages of rabbits have been associated with two rabbit subspecies: *Oryctolagus cuniculus algirus* and *Oryctolagus cuniculus cuniculus* (Branco et al. 2000; Carneiro et al. 2010). Only the *O. c. algirus* subspecies occurs in Continental Portugal (Branco et al. 2000; Delibes-Mateos et al. 2023; Díaz-Ruiz et al. 2023). As a result, this text will specifically refer to the Iberian rabbit, the common name of the *O. c. algirus* subspecies (Mira et al. 2023).

Given the fundamental role of the Iberian rabbit in Mediterranean ecosystems, its scarcity is a major concern. It acts as an ecosystem engineer (Jones et al. 1994; Gálvez et al. 2008), altering vegetation structure through grazing and seed dispersal, and providing refuge for other animal species that use its warrens (Willot et al. 2000; Gálvez-Bravo et al. 2009; Dellafiore et al. 2010; Bobadilla et al. 2023). Furthermore, the rabbit holds great significance as a game species in the Iberian Peninsula, where hunting holds high socio-economic importance (Paixão et al. 2009; Villafuerte et al. 1998). However, its primary contribution lies in being the main prey for numerous avian and mammalian predators, including endangered species like the Iberian lynx (*Lynx pardinus*) and the Spanish imperial eagle (*Aquila adalberti*) (Delibes and Hiraldo 1981; Delibes-Mateos et al. 2008a). Consequently, efforts to reinforce rabbit populations

are commonly incorporated into predator conservation programs (Moreno et al. 2004; Guil et al. 2014; Carro et al. 2019).

While successful rabbit restocking programs exist, even in areas where the species was absent, despite suitable habitat (Villafuerte et al. 2008; Guil et al. 2014), this outcome is unusual. There is an ongoing debate among hunters and conservationists concerning the effectiveness and high economic costs of restocking (Ferreira and Delibes-Mateos 2010; Guerrero-Casado et al. 2013a; Carro et al. 2019).

Releasing animals into a new habitat can be challenging and often results in high failure rates, especially during the first few days after release (Calvete et al. 1997; Letty et al. 2002; Calvete and Estrada 2004; Rouco et al. 2010). Early mortality can occur due to transportation and handling stress, adjustment to the new environment, exposure to local viruses and diseases, and predation (Calvete et al. 1997; Letty et al. 2000, 2002; Cabezas et al. 2011). Furthermore, this action presents significant risks for the resident population, including the possibility of sanitary or genetic contamination (Delibes-Mateos et al. 2008b).

Restocking failures often occur due to inadequate planning, which violates critical assumptions necessary for implementing proper management (Calvete et al. 1997; Calvete and Estrada 2004; Guerrero-Casado et al. 2013a). Despite available information on game management and restocking, many hunting estates, especially those without a game manager, still carry out unplanned restocking operations (Machado et al. 2017).

For successful rabbit restocking, careful planning of all restocking phases is essential. This involves selecting a donor population genetically similar to the native population (Delibes-Mateos et al. 2008b) and individuals in good body condition. Additionally, it is crucial to establish strict guidelines for transportation, handling, and vaccination to minimise initial stress on the rabbits, which can affect their physiological condition (Cabezas and Moreno 2007), increasing vulnerability to predation or diseases.

Based on practical research, protocols have improved, highlighting good practices and suggestions to increase restocking success. For instance, restocking should only be considered after ensuring the species' habitat requirements are fulfilled, promoting the rapid adaptation of the population relocated to the release area (Moreno and Villafuerte 1997; Cabezas and Moreno 2007; Cabezas et al. 2011; Guil et al. 2014).

Rouco and colleagues (2010) argued that it is important to prevent short-term dispersal. This is usually achieved by releasing the animals in fenced areas that serve as quarantine or acclimation sites. Doing so allows the animals to adapt to the new environment immediately after release when they are most vulnerable. This reduces post-release stress and

ensures immediate access to vital resources such as shelter, food, and water, and protection against predation (Calvete and Estrada 2004; Rouco et al. 2008, 2010; Cabezas et al. 2011; Machado et al. 2017).

A long acclimation period increases the initial breeding stock and overall restocking viability (Letty et al. 2008; Rouco et al. 2010). Restocking programs should also consider other factors such as release timing, sex ratio, and rabbit age (Cotilla and Villafuerte 2007; Guerrero-Casado et al. 2013a).

Several authors studied the survival and dispersal of restocked rabbit populations in the short term (Letty et al. 2002, 2008; Calvete and Estrada 2004; Rouco et al. 2010; Machado et al. 2017). However, the behaviour of a restocked rabbit population in the medium and long term after an extended confinement period is poorly understood (Guerrero-Casado et al. 2013a).

We carried out an Iberian rabbit restocking in southern Portugal. We considered various factors, as described in the literature, that promote the success of rabbit restocking. The primary aim of this work is to assess the survival and space use of a rabbit population released in a restocking park, a semi-natural enclosure.

Specifically, we aimed to answer the following questions: 1– How did the survival rate of the restocked rabbits vary over time? Were there differences in the survival of males and females, rabbits released in warrens *versus* rabbits released in the shrubs, and rabbits that died due to predation or other causes? We expect higher mortality during the first few days after restocking, due to stress or predation (Calvete et al. 1997; Letty et al. 2000, 2002), as rabbits explore the new environment. Additionally, due to the same reason, we expect higher mortality rates among dispersing animals after the passages are opened. We predict a higher mortality rate for rabbits released in the shrubs than those released in warrens because the formers are expected to have higher mobility to find an adequate place to settle (Kolb 1991). We expect a higher mortality rate in males than females due to males' greater locomotor activity (Donázar and Ceballos 1989). Additionally, during the breeding season, male aggression intensifies as they compete for and maintain high social ranks (von Holst et al. 1999).

2– Did the sex of the rabbits, the type of release location, and the time since restocking (before or after the opening of the restocking park passages) influence the mean and maximum distances travelled from the released sites (rabbits' dispersion), and home range and core area sizes? We anticipate that male rabbits would travel longer distances to explore the territory, leading to larger home ranges than females (Parer 1982). We predict rabbits released in warrens would adjust more quickly and travel shorter distances, resulting in smaller home ranges and core areas than those

released in shrubs. Warrens are fundamental for rabbit populations, providing refuge from predators and extreme climatic conditions, and playing a key role in rabbit reproduction and establishing social ties (Parer and Libke 1985; Kolb 1991). Additionally, we hypothesise that, after the passages are opened, the rabbits would travel longer distances from the release site and have bigger home ranges and core areas, assuming some rabbits would disperse and explore the habitat outside the restocking park.

Materials and methods

Study area

We conducted the study in a hunting estate in Monchique Natura 2000 Special Conservation Area (SAC) (PTCON0037) (Regulatory Decree n.º 1/2020 of 16th March), Southwest Portugal ($37^{\circ} 16' 33''$ N, $8^{\circ} 29' 27''$ W) (Fig. 1). Weather is characteristic of Mediterranean climates (Rivas-Martinez and Loidi 1999), with minimum and maximum mean temperatures of 5.6°C and 11.6°C in winter (January), and 17.3°C and 24.0°C in summer (July). Annual rainfall averages 925.5 mm (Monchique, 1984–2022; SNIRH 2023). The relief is undulating, with altitudes ranging from 30 to 180 m above sea level (CNA 1982), and the soils are poor, mainly dominated by incipient soils (IHERA 1999). The landscape is largely dominated by dense Mediterranean shrubs, occupying about 75% of the area, but eucalyptus (*Eucalyptus globulus*) plantations and agricultural fields (mostly cereal crops and orchards) can also be found, the last ones, in the valley of Odelouca river. Previous surveys indicated the presence of Iberian rabbits in the area, with scattered and low-density populations.

Restocking park, a semi-natural enclosure

We built a 1.7-ha restocking park to acclimate the restocked rabbits and prevent the entry of terrestrial carnivores (Fig. 2). A wire fence with a simple mesh of 40 mm, 1.8 m high, and 50 cm buried underground in an “L-shape”, delimited the park (Fig. 2b). This first fence was reinforced at the base with a second fence, 1 m high (15 mm mesh), with 50 cm below ground to prevent rabbits from getting through. Also, on the top of the fence, we added four lines of barbed wire. We installed under the fence 16 passages, made of plastic tubes 12.5 cm in diameter, to allow rabbit dispersion to the surrounding area (Fig. 2c).

No measures were taken to exclude aerial predators since the restocking was incorporated in the “Compensatory measures and specific monitoring of the Odelouca's Bonelli eagle couple, arising from the environmental impact

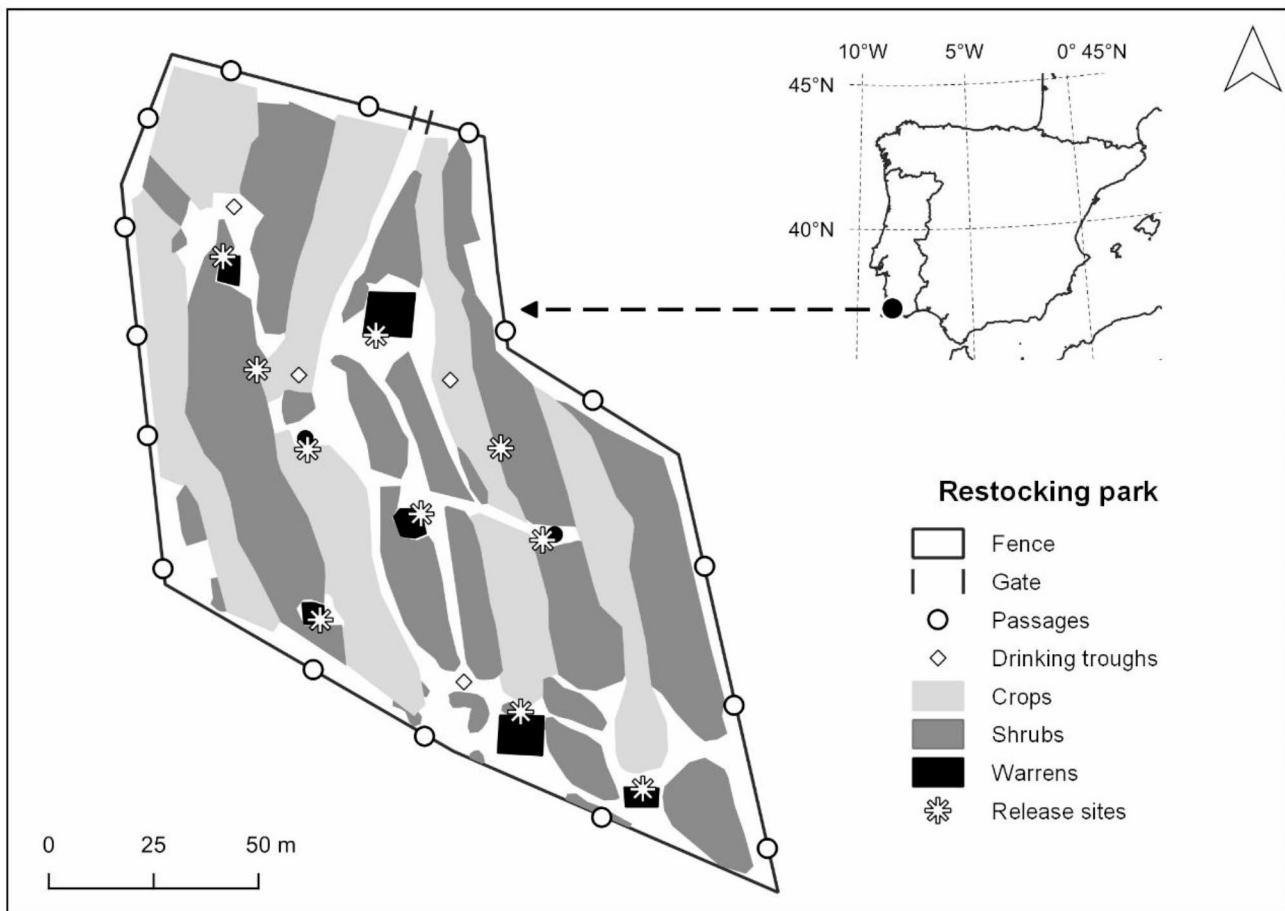


Fig. 1 Location and schematic representation of the restocking park in Southern Portugal. The release sites of the restocked rabbits are also marked

assessment process of the Sines-Portimão high-voltage power line" project (REN et al. 2009).

Inside the fenced park, we ensured food, shelter, and water availability (Figs. 1 and 2a). We alternated patches of herbaceous crops, a mixture of Leguminosae and Graminae (food), and patches of Mediterranean shrubs (shelter) and we installed four drinking troughs (water). Additionally, we built eight uniformly spaced (as much as possible) warrens, to increase the availability of shelter and breeding sites. We built four different types of man-made warrens, two of each: logs, Mayoral® – registered trademark, pallets, and tubes.

Rabbit release protocol

We conducted the restocking on 28th October 2007, using adult Iberian rabbits from a certified national breeder, who kept the animals in a semi-natural regime, i.e. living outside in enclosures but with food supplementation. We chose a breeder within the natural distribution area of the subspecies *O. c. algirus* (approximately 160 km from the study area) to ensure genetic similarity to the native populations (Branco

et al. 2000; Carneiro et al. 2010). Before travelling, the rabbits were fed and vaccinated against rabbit haemorrhagic disease (RHD) and myxomatosis.

75 Iberian adult rabbits, 19 males and 56 females (1:2.9 ratio) were transported overnight and arrived at the enclosure at sunrise (e.g., Machado et al. 2017), to minimise the effects of thermal stress, during animal transportation, manipulation and release. Additionally, releasing the rabbits early in the day, when they are less active and more likely to be resting, reduces their movement as they acclimate to their new environment in the initial hours after release. This makes them less vulnerable to stress and predation (Letty et al. 2008). With the sex ratio of 1:2.9, we intended to promote the formation of initial family nuclei with enough females for males, thereby minimising the risk of conflicts arising from competition for recruiting females for reproduction (von Holst et al. 2002).

We submitted all animals to a clinical examination, which preceded their release into the restocking park. Only healthy rabbits were released. Each rabbit was weighed, sexed, subjected to internal and external deworming, and



Fig. 2 Restocking park limits (a; white line), fence (b) and passages (c)

identified by a small, numbered metal tag placed in the left earflap. We performed all the procedures as quickly as possible to minimise the stress due to handling. A veterinary team oversaw all procedures, ensuring compliance with Portuguese legal regulations. All actions were done following the guidelines of the American Society of Mammalogists for the use of wild mammals in research (Gannon et al. 2007), and the guidelines for the treatment of animals in behavioural research and teaching (ASAB/ABS 2006)

and with the authorisation of the Portuguese Conservation Agency (ICNF).

We then released the rabbits in 10 locations inside the restocking park: inside each of the eight warrens and in two randomly selected locations in shrub patches (Fig. 1). At each location, we released groups of 5–8 individuals, maintaining approximately the same ratio of male/female.

To enhance rabbit survival during the initial weeks following restocking, we confined the rabbits within the fenced park by keeping the passages closed. This acclimation

period allowed the animals to adjust to the new environment before dispersing into the surroundings (Machado et al. 2017).

Initially, we planned an acclimation period of about four weeks. However, due to insufficient rain for the growth of autumn crops inside and outside the restocking park, we decided to postpone the opening of the passages. Throughout this period, we provided water (Fig. 1), and occasionally supplementary food (cereal seeds) near the four drinking troughs.

On 8th January 2008, after a confinement period of 72 days, we opened the enclosure passages to facilitate the natural dispersal of rabbits into adjacent areas following their acclimation to the new habitat.

Rabbit survey by radio-tracking

Out of the 75 rabbits released, we randomly selected 22 (11 males and 11 females), approximately 30% of the total released. These rabbits had radio transmitting collars (model: lpm-2700; weight \approx 25 g; Wildlife Materials, Inc., USA). The collars were designed to add no more than 5% of the animal's weight and to ensure no significant additional energetic costs (Wilson et al. 1996; Sikes et al. 2011). We considered the population wearing radio collars a representative sample of the entire population. The collars had an activity sensor, which generated a different pulse rate after 4 h of inactivity, ensuring we registered only locations of live rabbits. We released two radio-tagged individuals (one male and one female) in each warren, and three, in two randomly selected locations in the shrub patches (one male and two females in one location, and two males and one female in the other).

Monitoring occurred between 30th October 2007 (two days after restocking to allow rabbits to recover from transportation and handling stress; Teixeira et al. 2007), and 24th April 2008. We located the animals daily during the first two weeks after restocking and the first five weeks following the opening of the passages and about three times a week during the rest of the monitoring period.

For each animal, we evenly surveyed six different 4-h radio-tracking intervals covering a complete 24-h cycle (00:00–04:00, 04:00–8:00, 08:00–12:00, 12:00–16:00, 16:00–20:00, 20:00–00:00 h), so that specific daily patterns of activity would not bias radio locations (Villafuerte et al. 1993). Each tracking session started at least 12 h after the previous session to minimise autocorrelation in the radio-tracking data.

We used a TS-1 receiver (Telonics, Telemetry– Electronics Consultants, USA), and an external 3-element Yagi directional antenna (Wildlife Materials, Inc., USA) for tracking. The transmitters operated within the 150–152 MHz

frequency range. During each tracking session, we first located each radio-collared rabbit using the homing method. As we approached within about 10 to 15 m, we conducted multiple triangulations. This approach aimed to minimise any disturbance to the rabbits and reduce the margin of error for each location, which could increase if we relied solely on triangulation from a greater distance. A positioning measurement was recorded at each radio location using a handheld GPS. We monitored each radio-collared rabbit until its death or until the end of the battery life of the transmitter. The average duration of the monitoring period per animal was (\pm SE) 77.4 ± 14.8 days (range: 1–179).

Concerning survival analysis, when a collar emitted a signal of inactivity, the technician searched for the animal and identified the presumed cause of death. We also regularly checked the enclosure and the surroundings for signs of dead rabbits such as carcasses, body parts, ear tags, or tufts of hair. Whenever possible, the carcasses were collected and subjected to veterinary exams to determine the specific cause of death. We categorised the cause of death as predation or other causes. Other causes included dead rabbits with no visible signs of predation, non-recovered carcasses inside warrens, or other unknown reasons. Animals found dead on the n th day after release were considered to have survived $n - 1$ days (Rouco et al. 2010).

Statistical analysis

Survival

We calculated the finite survival rate by dividing the number of individuals alive at the end of the monitoring period by the number of individuals alive at the beginning.

We estimated survival rates for rabbits during the monitoring period with the Kaplan–Meier product limit estimator. This non-parametric technique shows the probability of survival over time (Pollock et al. 1989). Additionally, 95% confidence intervals were computed. We estimated the survival curves for the entire population, and separately for males and females, for rabbits released in warrens and rabbits released in shrubs, and for rabbits that died due to predation or other causes. We used a non-parametric log-rank test to compare survival curves between these pairs of groups. This test compares survival probabilities between two groups, with the null hypothesis stating the survival functions of the groups being compared do not differ.

We conducted the analysis using the “survival” package (Therneau 2023) in R software version 4.2.3 (R Core Team 2023).

Space use

We used radio-tracking data to calculate four space use indicators for each rabbit: maximum distance travelled from the release point, mean distance travelled from the release point, home range, and core area. The maximum distance travelled is the Euclidean distance from the release point to the furthest recorded point, while the mean distance travelled is the average Euclidean distance of all locations of each rabbit from the release point (Moreno et al. 2004; Machado et al. 2017). Home range and core area sizes were determined using 95% and 50% minimum convex polygon, respectively (MCP, Mohr 1947).

Only one rabbit dispersed from the restocking park to the surrounding area. Given the small sample size, we included this rabbit in the analysis. However, we omitted all its locations recorded outside the restocking park, focussing the analysis on the rabbit population within the restocking park.

We chose to use Minimum Convex Polygons (MCPs) for several reasons (Harris et al. 1990; Hemson et al. 2005; Devillard et al. 2008; Ziege et al. 2020):

1 - They are typically more robust when the number of locations is small.

2 - They are less affected by location density than Kernel or the k-nearest neighbours convex hull methods. Since rabbits inhabit holes, multiple relocations may occur at the same place, potentially causing a lack of convergence in the smoothing parameters.

3 - MCPs have also traditionally been the most common metric used in home range studies, allowing for easier comparisons with other studies.

In this case, we estimated a 95% MCP instead of a 100% MCP. We based this decision on the fact that MCPs are highly sensitive to the most extreme locations in space (Burgman and Fox 2003). By excluding 5% of extreme locations, we improve the standardisation in animal home range calculations. These extreme locations are believed to be more related to the dispersal or exploratory movements of the animals rather than the regular movements that typically define animal home ranges (Harris et al. 1990).

The minimum number of fixes needed for reliable home range estimation was determined by analysing incremental-area plots (Odum and Kuenzler 1955; Harris et al. 1990; Kenward 2001) using R software version 4.2.3 (R Core Team 2023). We considered the number of locations satisfactory for MCP estimation when the relationship between MCP percentages of the total area and the number of fixes reached an asymptote. This refers to the point at which additional locations result in a minimal increase in range size. Defining where asymptotes begin and determining the number of locations required is subjective, so we adopted a criterion of less than a 10% increase in the area to identify

the asymptote (e.g., Hayward et al. 2009; Plotz et al. 2016). We only included rabbits with enough locations above this threshold for further analysis.

We used incremental-area plots to estimate the average number of days it took for the rabbit home ranges to stabilise after restocking.

For each of the four space-use indicators, we computed three values for each animal: one for all the locations acquired during the monitoring period, and two by dividing the locations obtained before and after the opening of the passages.

Distance indicators were calculated using QGIS (QGIS Development Team 2023), and area indicators were determined in R software version 4.2.3 (R Core Team 2023) using the “adehabitatHR” package (Calenge 2006).

Due to the small sample size, we used non-parametric statistical tests to compare the space use indicators, determined for the entire monitoring period, for male and female rabbits, and rabbits released in shrubs versus those released in artificial warrens. We employed the Two-sample Mann-Whitney U test for independent samples for this comparison. For the same reason, we compared the space use indicators for locations registered before and after the opening of the passages using the non-parametric Wilcoxon signed-rank test for related samples.

We also evaluated rabbit site fidelity in QGIS, by calculating the percentage of overlap between the home ranges and core areas of each rabbit in two different periods: before and after the opening of the passages (Jerosch et al. 2017; Wereszczuk and Zalewski 2019). The percentage overlap was determined using the formula $(A_{ij}/A_i) \times 100$ where A_{ij} represents the area of overlap between the areas at time i (before) and time j (after), and A_i represents the area in time i (before the opening of the passages) (Jerosch et al. 2017).

Results

Throughout the study, we recorded 864 rabbit radio locations. On average, each rabbit had 39.1 ± 7.4 locations (range: 1–89). The tracking period for each rabbit varied from 2 to 179 days, with an average of 77.4 ± 14.8 days (Table 1).

Survival

During the monitoring period, we documented 14 mortality events (Table 1). Only eight of the 22 radio-collared rabbits survived (four males and four females), representing an overall finite survival rate of 36.4%.

Predation was the main cause of death. Of the 14 rabbits found dead, nine exhibited signs of predation (64.2%),

Table 1 Details of the 22 radio-tracked rabbits. For each rabbit, we provide detailed information about sex, release site, number of locations, number of tracking days, rabbits that stabilised home ranges (HR, marked with x), and rabbits with enough locations before and after the opening of the restocking park passages (marked with x)

Rabbit ID	Sex	Release site	Nº of locations	Nº of tracking days	Death cause	Stable HR	Before and after locations
1	Male	Warren	3	6	Other		
2	Male	Warren	25	55	Other	x	
3	Female	Warren	7	10	Predation		
4	Male	Warren	1	3	Predation		
5	Female	Warren	18	37	Predation		
6	Male	Warren	26	62	Predation		
7	Male	Shrubs	84	177	-	x	x
8	Male	Warren	80	163	-	x	x
9	Male	Warren	5	8	Predation		
10	Female	Shrubs	1	2	Other		
11	Female	Warren	12	17	Predation		
12	Female	Shrubs	87	179	-	x	x
13	Female	Warren	28	71	Predation	x	
14	Female	Warren	89	179	-	x	x
15	Female	Warren	32	75	Other	x	
16	Male	Warren	87	174	-	x	x
17	Female	Shrubs	81	174	-	x	x
18	Male	Warren	25	52	Other		
19	Female	Shrubs	83	170	-	x	x
20	Male	Shrubs	12	22	Predation		
21	Female	Warren	1	3	Predation		
22	Female	Warren	77	64	-	x	x

particularly by birds of prey (Calvete et al. 1997; Lombardi et al. 2003). Indeed, during the monitoring period and near the restocking park, we heard vocalisations of a Eurasian eagle owl (*Bubo bubo*), and we found feathers and regurgitated pellets of this species with metal tags identical to the ones we placed on the rabbits' earflap. Additionally, we found two radiotelemetry collars near a Eurasian eagle owl nest, located about 250 m away from the restocking park. The remaining five rabbits died from other causes, two of them during the first week after restocking and three between the middle of December 2007 and the middle of January 2008.

Additionally, we found eight more carcasses or body parts of rabbits (six females and two males) within the restocking park. All were from the individuals we released, and all occurred within the first three weeks after restocking. Four of these carcasses showed signs of predation, while the other four rabbits died from different causes during the initial week after restocking.

The veterinary team conducted a lab examination on the six carcasses showing no signs of predation, found in the first week after restocking and including two radio-collared rabbits. The results revealed the cause of death may be related to a severe parasite infection, including Helminth (Cestoda, Nematoda) and coccidian.

Regarding rabbits that died more than a month after restocking, lab results revealed both radio-collared males

died of hyper-parasitism. As for the other rabbit, despite necropsy examinations suggesting sudden death due to intoxication, the veterinary team remained unable to determine the exact cause of death.

We found no evidence of death caused by myxomatosis or rabbit haemorrhagic disease during the monitoring period.

Survival probability for this population stabilised 74 days after restocking (Fig. 3). The estimated median survival time was 57.5 days, which means that on average, 50% of the population had died by that point. The probability of survival beyond 74 days was 35.4% (95%CI = 19.9 – 62.9%). Assuming the radio-collared population is representative of the total restocked population, we can estimate there were approximately 27 rabbits alive ($75 \times 0.354 = 26.6$; 95%CI = 14.9 – 47.2 rabbits) at the end of the radiotelemetry, out of the 75 restocked.

We found no significant differences in survival rates between females and males ($p=0.653$; Supplementary information: Fig. SI1) or between rabbits released in a warren and rabbits released in shrubs ($p=0.151$; Supplementary information: Fig. SI2). Furthermore, we have no evidence to suggest survival rates differ between rabbits that died due to predation or other causes ($p=0.484$; Supplementary information: Fig. SI3).

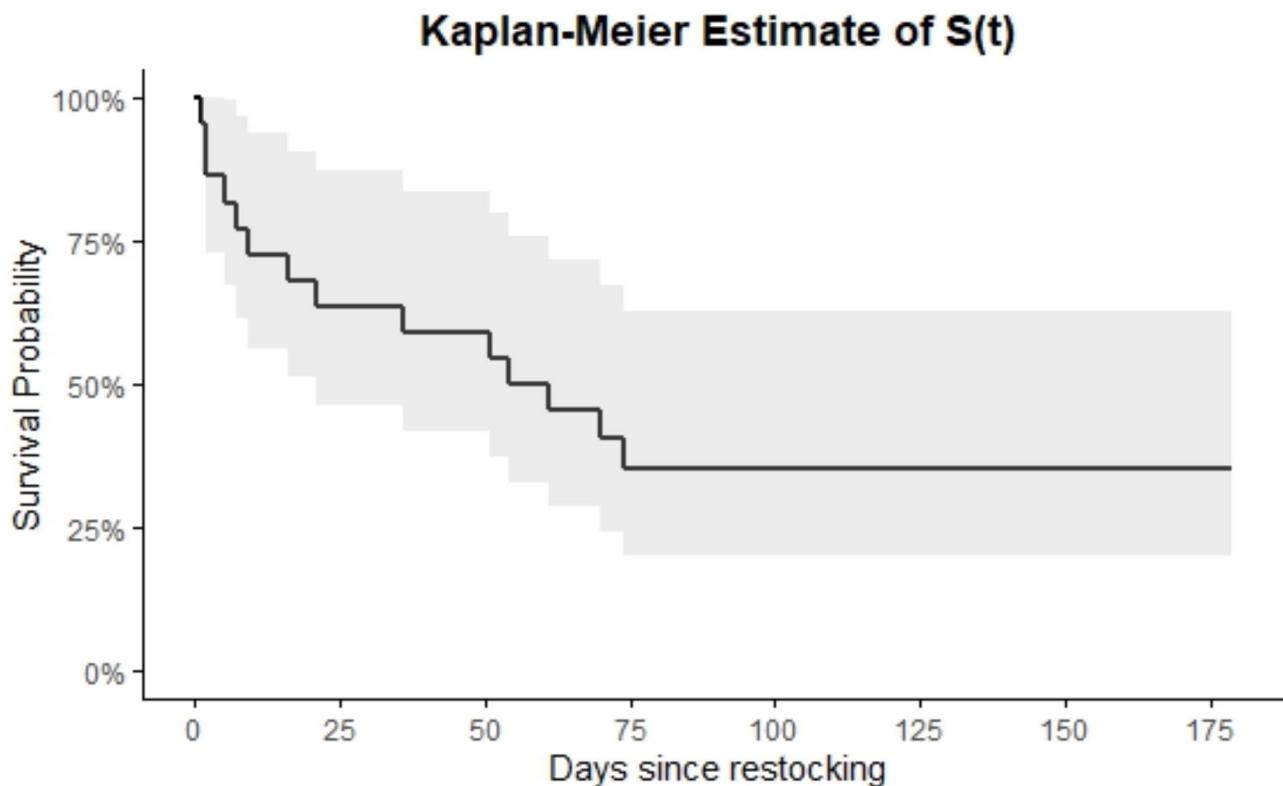


Fig. 3 Restocked rabbit survival probability curve during radio tracking, in a semi-natural enclosure, based on Kaplan-Meier estimate, with a 95% confidence interval

Space use

Eleven rabbits stabilised their home range (Table 1). On average, this restocked population took $96.364 \pm \text{SE } 12.615$ days to stabilise home ranges.

Only 8 rabbits had enough locations to compute space use indicators before and after the opening of the passages (Table 1). Before and after core areas overlapped on average $39.503 \pm \text{SE } 14.143\%$ ($n=8$), and home ranges overlapped on average $69.709 \pm \text{SE } 8.578\%$ ($n=8$).

Regarding space use indicators, the mean maximum distance travelled, and mean distance travelled were $81.304 \pm \text{SE } 8.097$ m ($n=11$) and $40.289 \pm \text{SE } 5.420$ m ($n=11$), respectively. Rabbits' home range size was on average $0.228 \pm \text{SE } 0.037$ ha ($n=11$), and rabbits' core area size was on average $0.055 \pm \text{SE } 0.009$ ha ($n=11$). We found no significant differences in space use indicators between males and females.

Only the core areas, determined for the entire monitoring period, of rabbits released in artificial warrens were significantly smaller ($p=0.042$) than those released in shrub patches (Fig. 4, Supplementary information: Table SI1). The average core area of rabbits released in warrens was less than half the size of those released in shrubs (warrens

-0.040 ± 0.009 ha, $n=7$; shrubs $-0.082 \pm \text{SE } 0.012$ ha, $n=4$).

Maximum distance travelled was the only space use indicator significantly higher ($p=0.023$) after the opening of the restocking park passages. Before the opening of the passages, the maximum distances travelled by rabbits from their release site were on average $76.280 \pm \text{SE } 5.016$ m ($n=8$), and after the opening of the passages $93.190 \pm \text{SE } 5.871$ m ($n=8$) (Fig. 5, Supplementary information: Table SI2).

Discussion

In the southern regions of Europe, conservationists and hunters often invest in rabbit restocking or translocation to areas where their numbers are declining. However, these efforts are sometimes unsuccessful (Calvete et al. 1997; Letty et al. 2002) and require significant economic costs (Ferreira and Delibes-Mateos 2010). Therefore, it is essential to assess all aspects of restocking efforts to maximise their effectiveness.

Furthermore, most studies involving rabbit restocking or translocations tend to concentrate on the first few weeks after the animals are released (e.g. Letty et al. 2002; Letty

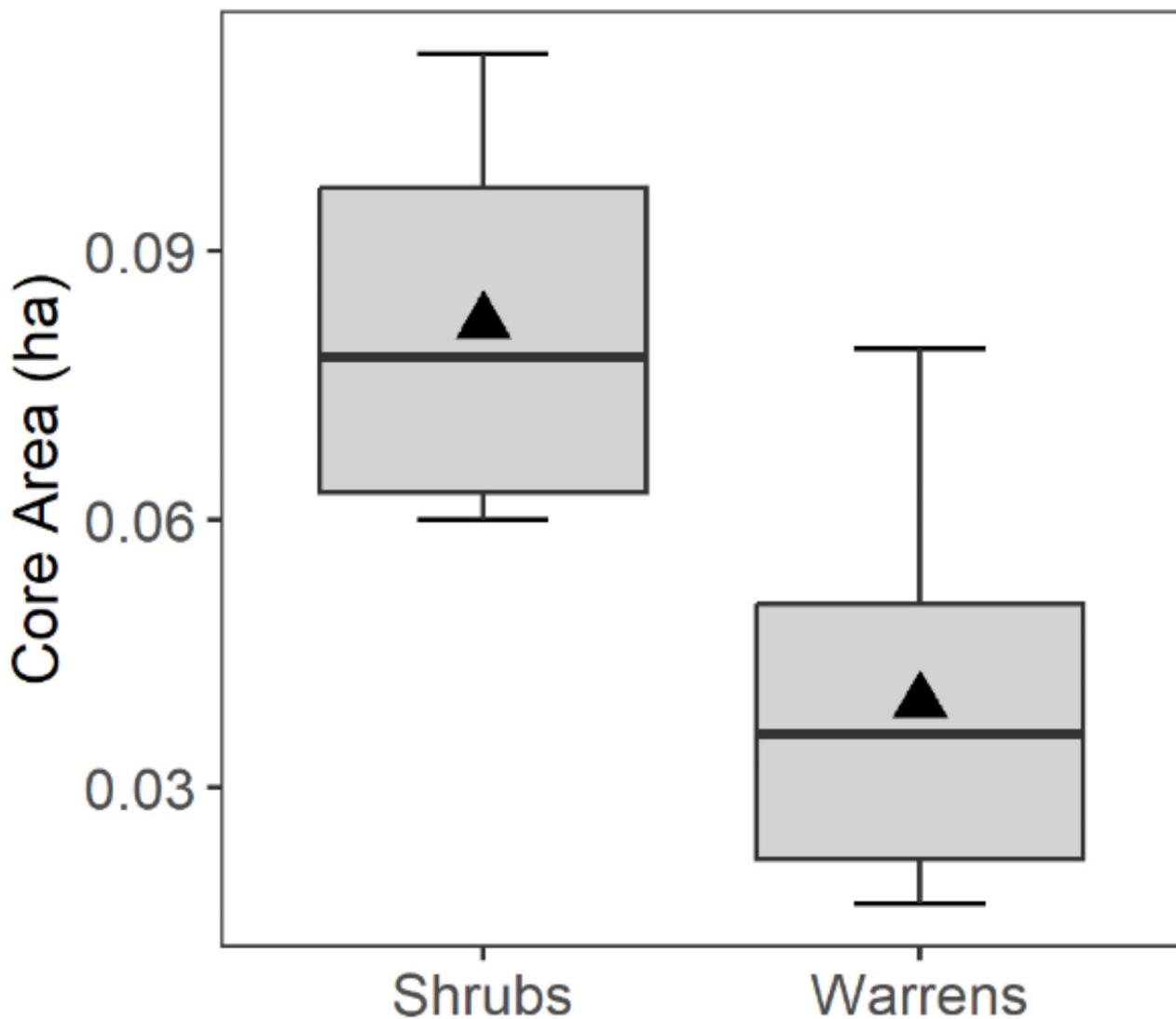


Fig. 4 Core area size (50% minimum convex polygon) of the restocked Iberian rabbits, determined for the entire monitoring period, according to the type of site they were released, shrubs, or warrens. In each boxplot of the graph, the triangle is the mean, and the darker horizontal

line is the median. The length of the box represents the interquartile range (i.e., the difference between the 75th and 25th percentiles). The dots outside the whiskers are outliers

et al. 2003; Calvete and Estrada 2004; Rouco et al. 2008; Rouco et al. 2010) and available data on the long-term outcomes for the restocked rabbits is scarce (e.g., Cabezas et al. 2011; Machado et al. 2017; Tobajas et al. 2021).

In this study, we assessed the long-term outcomes by monitoring the survival and space use of an Iberian rabbit population released in a restocking park, a semi-natural enclosure, for six months. In this permanent enclosed area, surrounded by a fence, released rabbits could roam freely, and the habitat closely resembled the surrounding environment. The purpose of the enclosure was to facilitate rabbit settlement by reducing mortality from terrestrial predators

and post-release stress, particularly during acclimation. Additionally, the restocking park aimed to establish a local breeding core of rabbits that expands naturally to the surrounding areas (Rouco et al. 2008; Guerrero-Casado et al. 2013b).

We implemented the rabbit restocking protocol considering important issues that contribute to the success of rabbit restocking. For example, we ensured the restocking park fulfilled the habitat requirements for the species, namely, in terms of food, shelter and water (Cabezas and Moreno 2007; Letty et al. 2008), selected a donor population genetically close to the native rabbit populations (Delibes-Mateos

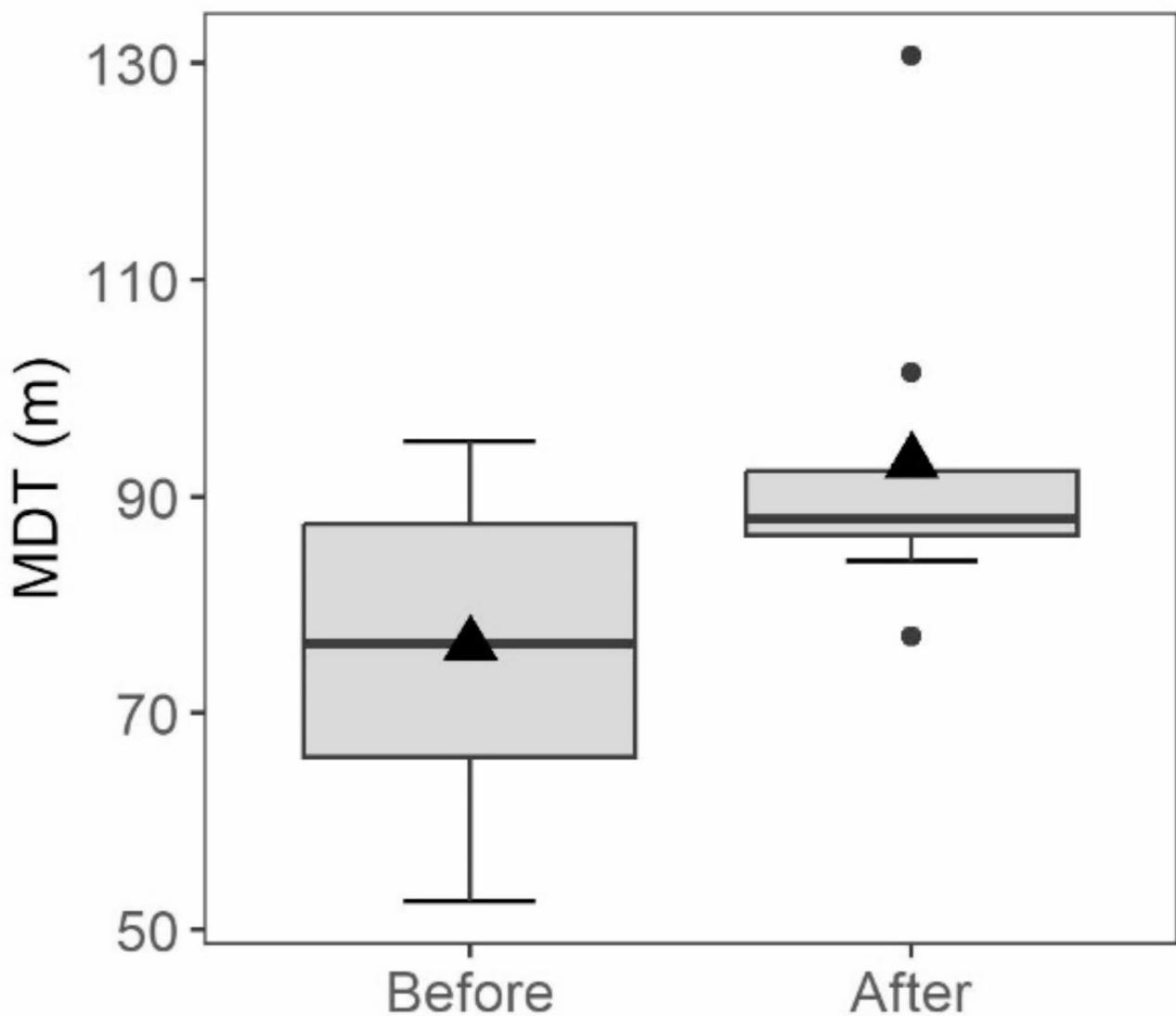


Fig. 5 Maximum distance travelled (MDT) by the restocked Iberian rabbits, before and after the opening of the restocking park passages. In each boxplot of the graph, the triangle is the mean, and the darker

horizontal line is the median. The length of the box represents the interquartile range (i.e., the difference between the 75th and 25th percentiles). The dots outside the whiskers are outliers

et al. 2008b), established strict guidelines for transportation, handling, and vaccination, and implemented a soft-release protocol with a long confinement period (Rouco et al. 2010).

Our findings should be interpreted cautiously due to the limitations of this study and cannot be extrapolated to natural populations. Our research was confined to a single restocking park, with only 1.7 ha, that may influence rabbit movements and home ranges. Moreover, the limited number of rabbits could increase the risk of statistical errors, particularly when the sample is divided to make subgroup comparisons. Grouping the data could increase statistical power, allowing for more robust and reliable conclusions.

However, we could risk losing statistically relevant distinctions between subgroups, such as those we attained.

Nevertheless, our results provide meaningful preliminary insights, highlighting important issues to consider for enhancing the success of rabbit restocking programs.

We found that rabbit survival was globally low and that space use only presented punctual differences among rabbits released in shrubs and warrens and between before and after the opening of the restocking park passages.

Survival

Despite implementing a restocking protocol that included measures known to enhance restocking success, and the Iberian rabbits being released in a semi-natural enclosure, at the end of the monitoring period (179 days), only eight out of the 22 radio-tagged rabbits survived, reflecting a finite survival rate of 35.4%.

This survival rate aligns with findings from several studies on rabbit restocking, including those by Letty et al. (2002), Drees et al. (2009), and Tobajas et al. (2021). However, in these studies, rabbits were released in unfenced areas without undergoing a quarantine period before release. Calvete and colleagues (1997) also found similar survival rates. In their study, the rabbits were released in unfenced areas after a two-week confinement period.

On the other hand, other studies, where rabbits also underwent a period of confinement before release, but were released in open areas, contrary to our study, estimated higher survival rates. Moreno and colleagues (2004) evaluated the success of introducing rabbits for predator conservation in Spain. After a 90-day study period, researchers reported survival rates between 70% and 78% for the introduced rabbits, depending on the season in which they were restocked. Rouco and colleagues (2010) estimated a survival rate between 79% and 87% in a study aiming to assess the effect of different confinement periods on the survival of translocated rabbits. However, the authors only monitored the radio-collared rabbits for 10 days.

The survival rates stabilised 74 days after restocking, immediately after the opening of passages on the 72nd day. We had anticipated increased mortality of dispersing animals after the opening of passages, but the results do not support this. This outcome, combined with limited dispersion, suggests the surviving animals have adapted well to the habitat in the restocking park. In this respect, the enclosure partially fulfilled its purpose.

Based on field observations, we found radio-collared rabbits primarily died due to predation by birds of prey, most likely caused by a Eurasian eagle owl (*Bubo bubo*). We believe the sudden release of many rabbits in the small area of the restocking park, attracted the owl, leading it to kill more rabbits than needed. This type of behaviour is known as multiple predation or surplus killing (Short et al. 2002). Other authors observed similar predator behaviour following rabbit translocations (Calvete et al. 1997; Moreno et al. 2004; Cabezas et al. 2011; Guerrero et al. 2013b). We found no signs of predation by terrestrial mammal carnivores, suggesting the structure of the restocking park fence was robust enough to prevent the entry of carnivorous mammals.

In the first few weeks after restocking, the mortality rate of rabbits due to causes other than predation, which

are usually associated with induced stress (Calvete et al. 1997; Letty et al. 2000, 2002), was relatively low. We only observed two deaths among the radio-collared rabbits in the first week, which represents 9.1% of the radio-collared population. Other studies have also reported low rabbit mortality rates due to stress, which were lower than the mortality rates caused by predation (Calvete et al. 1997; Calvete and Estrada 2004; Drees et al. 2009). We believe the transport and handling methods were appropriate, and the requirements for the animals to acclimatise were met within the enclosure, which helped minimise post-release stress.

The veterinary analysis showed these two rabbits died due to severe parasite infections, even though the initial exams performed on the 1st day after restocking confirmed the low degree of parasitic infection of the animals. This confirms that stress can cause strong immunosuppression in rabbits, making them vulnerable to developing severe parasitism even from low initial infections (Letty et al. 2000).

After these first few weeks of restocking, an additional three rabbits were found dead without any signs of predation. These late deaths cannot be attributed to adaptive stress since they occurred after a considerable amount of time had passed. Post-mortem examinations were conducted, and the veterinary team was unable to determine the exact cause of death for one of the rabbits. However, lab results confirmed that the other two rabbits, both males, died due to hyperparasitism. There could be several reasons behind this diagnosis. One possible cause is the absence of basic nutritional requirements within the restocking park. As mentioned earlier, in the year of the restocking, the lack of rain caused a delay in the germination of crops, both inside and outside the restocking park. Although we occasionally provided supplementary food during acclimation, its quantity and quality may have been insufficient. The shortage of green biomass, rich in nutrients and water content, could have weakened some individuals (Dudzinski and Myktyowycz 1960; Alves and Moreno 1996). This could have made them more susceptible to parasitism, leading to mortality even outside the critical period of adaptation (Villafuerte 1994; Reglero et al. 2007). Increased parasitism may also be caused by intra-specific aggression associated with male social interactions during the reproductive season (von Holst et al. 1999). During this time, the stress caused by the high rabbit concentration inside the restocking park, with rabbits competing for territory and mating partners, can weaken their immune systems and make them more susceptible to parasitic infections (von Holst et al. 1999).

We initially expected that males would have a higher mortality rate than females. However, our results did not support this hypothesis. Several authors also noted the absence of differences between sexes in survival after release (Calvete et al. 1997; Letty et al. 2003; Calvete and Estrada 2004).

Furthermore, our results revealed no significant difference in the survival rates of rabbits that died due to predation and those that died from other causes.

Although the mortality of rabbits released in shrubs was not significantly higher than those released in warrens, as we expected, we did notice a slight tendency to higher survival in rabbits released in artificial warrens (Supplementary information: Fig. SI2). Warrens are essential in maintaining healthy rabbit populations as they serve as breeding sites and provide refuge from predators (Parer and Libke 1985; Kolb 1991). Natural burrows are challenging to excavate in the study area due to the rocky and hard soil (IHERA 1999). Therefore, we believe the rabbits released in shrubs exhibited more ranging behaviour to find suitable refuge, making them vulnerable to predation. On the other hand, rabbits released into warrens settled within them and had less need to explore the area.

Space use

The radio-collared rabbits stayed inside the restocking park, except for one male rabbit who dispersed and settled about 200 m north of the release location. We believe the carrying capacity of the restocking park was not reached due to the mortality that occurred before the passages were opened. As a result, the surviving rabbits established themselves within the park without feeling compelled to explore the nearby areas. Right after restocking, rabbit density within the park was high, at 44 rabbits per hectare. By the end of the monitoring period, considering the final survival rate of the radio-tracked rabbits, we estimated a density of 16 rabbits per hectare. This estimate does not account for the number of rabbits entering or leaving the park, as well as the number of newborn rabbits, due to the inability to accurately estimate those numbers.

Rabbits took about 90 days to stabilise their vital areas, in contrast to the 8.3 days reported by Moreno and colleagues (2004). This delay may have been caused by factors such as a lack of nutritious food, aggressive interactions, or the pressure of predation, which can all contribute to erratic behaviour.

Rabbits' home range (HR) and core area (CA) size were relatively smaller than what is reported in the literature (e.g., HR 6.3 ha Hulbert et al. 1996; HR– 2.1 ha Moseby et al. 2005; HR 1.2 ha and CA 0.4 ha Devillard et al. 2008; 9.6 ha and CA 1.3 ha - Rouco et al. 2019), which is attributed to the fact that rabbits with radio collars remained within the restocking park.

After the acclimation period, the maximum distances travelled by rabbits significantly increased. However, there was a high overlap between their home ranges before and after the opening of the restocking park. This suggests the

surviving rabbits settled within the park and explored the nearby areas around their home ranges when the passages were opened instead of dispersing to other locations.

As we hypothesised, animals released within warrens had significantly smaller core areas than those released in shrubs. This indicates concentrated activity within and around the warrens. On the other hand, animals released in shrubs exhibited more ranging behaviour, which makes them more vulnerable to predation (Parer and Libke 1985; Kolb 1991).

Contrary to our predictions, our study found no significant differences in spatial indicators between males and females. This result aligns with the findings of Rouco and colleagues (2019), who reported similar results regarding home range and core area sizes, and Letty and colleagues (2008), who reported no difference in distance from release warrens between males and females. These findings suggest separate management measures for males and females may be unnecessary for restocking efforts.

Management suggestions

Based on our research findings, we believe we can make recommendations to improve rabbit restocking success in similar operations.

The fence we installed in the semi-natural enclosure prevented terrestrial predators from entering. Protection against predators, namely terrestrial, is essential to ensure the investment in restocking does not merely result in an immediate and short-term food supply for predators. Therefore, we recommend that conservationists and hunting managers involved in rabbit recovery release rabbits preferably in confined, fenced areas. This helps prevent rabbit dispersion and protect them from predation by terrestrial mammals during the initial days after release.

The enclosure effectively protected against predation by terrestrial mammals, but it failed to provide adequate protection from birds of prey. Predation by birds of prey was the main cause of rabbit mortality, especially in the first few weeks after release. We took no measures to exclude aerial predators since the restocking was part of the “Compensatory measures and specific monitoring of the Odelouca’s Bonelli eagle couple, arising from the environmental impact assessment process of the Sines-Portimão high-voltage power line” project (REN 2009). The results clearly showed this decision was incorrect. We believe the released rabbit population should be protected from both mammalian predators and birds of prey, at least until the animals are adapted to the new environment. Avoiding predator activity in the release area during the critical period could substantially increase rabbit survival (Calvete et al. 1997).

To prevent predation by birds of prey, a soft net could be installed to cover the top of the restocking park, especially during the acclimation period (Guerrero-Casado et al. 2013b). However, this approach may be expensive and logically challenging to implement in large-scale restocking parks like ours. It is also advisable to avoid installing fences near large trees that could serve as perches for diurnal or nocturnal birds of prey during their hunting activities.

Considering our findings regarding space use, we recommend releasing rabbits into warrens that offer protection from predators in the most critical period (Parer and Libke 1985; Kolb 1991). This will help animals acclimate faster, reduce post-release stress, minimise distances to explore the new and unfamiliar environment, and ultimately decrease predation risk.

After restocking, we suggest long-term monitoring of the released rabbit population thoroughly and continuously for dispersal, mortality, and breeding success. This will allow the implementation of adaptive management and to take the actions needed to enhance the rabbits' survival and the overall success of the restocking effort whenever necessary.

Finally, given the limitations of our research, we acknowledge the need for future research with larger sample sizes to support the conclusions presented in the study and increase the replicability of these measures. Even though we know that the high costs associated with this type of action may always limit what can be done.

Conclusions

Our study presents findings on the survival and space use relevant to the recovery plans for the Iberian rabbit populations, based on releasing animals within a semi-natural enclosure and following specific guidelines.

We believe that our findings provide meaningful preliminary insights, even if considering the local context and the limitations of this study. Our management recommendations can be applied to similar restocking programs involving the release of rabbits in semi-natural enclosures in the southern Iberian Peninsula. These results and conclusions can be helpful for game managers and conservationists, given the importance of conducting restocking efforts to help recover this declining species that plays a fundamental role in Mediterranean ecosystems.

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Author contributions C.E., P.P., P.C.A. and A.M. conceived the idea and designed the methodology. C.E., H.S.M. and P.P. collected the data. C.E. analysed the data and wrote the first manuscript draft. All authors revised the manuscript, contributed with supportive information, and gave final approval for publication.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval Animal transportation and handling were in conformity with Portuguese legal regulations. All applicable international, national and institutional guidelines for the care and use of animals were followed.

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