Title: A longer confinement period favors European wild rabbit (*Oryctolagus cuniculus*) survival during soft releases in low cover habitats

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Abstract

Rabbit restocking is one of the most-used techniques in Spain carried out for conservation and/or hunting purposes. However, the success of rabbit restocking is generally low, thus many studies have assessed ways to reduce this problem, one of which is the use of a "soft release" procedure, whereby rabbits are acclimated to their release site for a variable time period prior to release. This study assesses the short-term effects of two soft release confinement periods on the survival of rabbits during an experimental restocking program carried out in southwest Spain. The survival rate of rabbits confined at the release site for six nights was significantly higher than those confined for a shorter period (three nights). The longer acclimation period after rabbit translocation minimized mortality while rabbits adapted to their new environment.

Keywords: confinement period, rabbit conservation, radio-tracking, restocking, soft release, translocation

Introduction

The wild rabbit (*Oryctolagus cuniculus*) is a keystone species in its original distribution being the staple prey of more than 30 predators (Delibes-Mateos et al. 2008a), moreover it is one of the most important small game species in Spain (Angulo and Villafuerte 2004).However, rabbit populations have declined dramatically in the Iberian Peninsula over the last 50 years, mainly by two viral diseases: myxomatosis and rabbit hemorrhagic disease (Moreno et al. 2007). In addition, human-induced habitat changes (i.e. intensification of agriculture and habitat fragmentation) have accelerated their decline, or local extinction, in many regions during the last century (Moreno and Villafuerte 1995).

As a result of the decline in rabbit populations, a variety of management measures have been implemented in recent decades to enhance rabbit recovery. Amongst these, rabbit restocking has been increasingly used, particularly in central–southern Spain, and from 1993 to 2002 thousands of rabbits were restocked in almost half of the hunting estates (Delibes-Mateos et al. 2008b). However, both scientific studies and managers' experience show that the success of restocking is generally low (Calvete et al. 1997; Letty et al. 2002;). High rabbit mortality during the ten days immediately following release appears to be the main limiting factor in rabbit restocking (Calvete et al. 1997). High initial mortality limits restocking success because it reduces the breeding stock and consequently the viability of the population (Letty et al. 2008). This high mortality can be related to stress, social factors, etc.. Between the different stages that can appear during translocation programs, capture, captivity, transportation and release are the most stressful (Teixeira et al. 2007). Many studies have assessed ways to reduce this problem, one of which is the use of “soft release”, whereby rabbits are acclimated to their new environment in mammal holding pens

(Jefferies et al. 1986; Short et al. 1992). Comparison of "soft" and "hard" (without an acclimation period) release methods have generally demonstrated improved survival and behavior benefits with soft releases (Bright and Morris 1994).

Different acclimation periods for wild rabbits have been applied in both natural (Calvete and Estrada 2004) and artificial warrens (Letty et al. 2000). However, no study has been carried out to determine the best acclimation period and its efficacy in increasing rabbit survival. We hypothesized that a longer confinement period would favor rabbit acclimation to their release site, decreasing the novelty environmental effect. Therefore, this study assesses the effect of two different confinement periods on the short-term survival of rabbits translocated to artificial warrens as part of a soft release process.

Materials and methods

Study area

The experiment was conducted in one of four restocking plots in the compensatory ecological area of Los Melonares (south of the Sierra Norte Natural Park of Seville, SW Spain; Fig. 1a). This region has two main biotopes, Mediterranean grassland (70%) and scrubland (30%). Rabbit abundance was relatively low before restocking, but both mammalian and raptor predators were present (Rouco et al. 2008).

The translocation site consisted of a grassland field approximately 4 ha in size, where artificial rabbit warrens were built (Rouco et al. 2008). Water and commercial pellet food suppliers were situated close to each warren and available *ad libitum* (Fig. 1b). Each artificial warren was surrounded by a wire net fence (warren pen), embedded 50 cm into the ground and extending 100 cm above ground; each pen had three to five rabbit doors (Fig.1c). The warren pens were aimed primarily to reduce immediate dispersal of rabbits while the pen doors were closed, and to facilitate acclimation. The

confinement period was defined as the time that the warren pen remained closed. Food and water were supplied *ad libitum* inside each warren pen during the whole confinement period, being administrated daily at daylight to avoid unnecessary disturbance of rabbits and terrestrial predators (mainly nocturnal).

Experimental design

To assess the effect of different confinement periods on the survival of translocated rabbits, we randomly selected 38 of the 181 rabbits introduced to the translocation site for monitoring following release. Each of these rabbits was fitted with a radio-collar (approximately 25 g; BIOTRACK, Wareham UK). The 38 radio-collared ("tagged") animals were distributed in two groups for release after a confinement period with two different duration ("release treatment groups"). For one treatment group, 15 (6♂, 9♀) rabbits were confined in the warren pen for three consecutive nights, and the pen doors were opened on the fourth day. For the other group, 23 (10♂, 13♀) rabbits were confined for six nights, and the pen doors were opened on the seventh day. Both tagged and untagged rabbits were released inside the artificial warrens. Thus, 2-5 tagged rabbits were released in each of 15 randomly selected warrens. The average number (±SE) of rabbits (tagged and untagged) per warren in the plot was 10.05±1.74. All rabbits were released to the warren pens within 24 h of being captured on a hunting estate approximately 300 km from Los Melonares. None of the released animals were vaccinated against viral diseases (myxomatosis and rabbit hemorrhagic disease).

Survival of rabbits

All tagged rabbits were tracked daily during the confinement period, and in the ten days following the opening of the warren pen doors. Tracking to determine their position and whether they were dead or alive was done in daylight. Causes of death

were determined by examining rabbit carcasses, identifying bite marks on the body and radio collar, examining the location of the remains of rabbits, and other signs. Predation was assigned to terrestrial carnivores when incisor marks on collars could be identified, or when scats, rabbit caecum or buried and half-buried corpses were found. On the other hand, rabbit assigned to predation by terrestrial carnivores could also be scavenged. Predation was assigned to raptors when evidence including feathers, characteristic tufts of torn-out fur, or remains of long bones were found. Deaths included in the "other causes" category included those assigned to scavenged, disease, and causes related to handling stress or aggression associated with social interactions (Calvete and Estrada 2004; Moreno et al. 2004). Deaths inside warrens were also included in this category because it was impossible to recover the corpses. Animals found dead on the n-th day after release were considered to have survived n-1 days.

Data analysis

Survival and mortality rates, 95% confidence limits (c.l.) were calculated and compared using the *Z* statistic using MICROMORT and following the recommendations described by Heisey and Fuller (1985). Two-tailed *Z* test was used to test differences between survival of the two treatment groups as a function of the confinement period. Because of the different confinement period lengths, we compared daily survival rates between the two treatment groups during this first period (confinement period). To check for differences in survival between the two treatment groups during the critical period (following ten days after confinement period), cumulative survival rates ten days after the confinement period were compared.

Results

Most of the tagged animals survived the confinement period. However, one tagged animal in the three-night confinement period treatment pen died during the

confinement period. This animal, found with the radio collar in its mouth, was not included in our analyses (n = 37 tagged animals).

Of the 14 tagged rabbits that were confined for three nights inside the warren pen, two died during the confinement period; one was predated by a red fox (*Vulpes vulpes*) and the other one had no signs of predation (assigned to handling stress). In the six-night treatment, three animals were found dead during the confinement period. Two were found inside the warren, and another was predated by a red fox. Daily survival rates were high for both confinement periods (0.86, c.l. = 0.694–1, for the shorter confinement period; 0.89, c.l. = 0.772–1, for the longer period) but not significantly different (Z = 0.42; P = 0.676). Survival rates of females and males during the confinement period did not differ significantly between the two treatment groups (three nights: Z = 0.23, P = 0.180; six nights: Z = 0.28, P = 0.222).

The cumulative survival rate for the ten days following the opening of the warren pen doors was estimated for each treatment. Rabbits that were confined for six nights had significantly higher survival rates than those confined for three nights (Z = 2.06, P = 0.039; Fig. 2).

Most deaths occurred during the days immediately following the opening of the warren pen doors, and mainly in the group with the shorter confinement period. Deaths in the group of animals from the longer confinement period treatment occurred throughout the following ten days (Fig. 2). Predation by terrestrial carnivores was the main cause of death during the study. After release, rabbits that were maintained for the shorter confinement period were more frequently predated (mortality rate due to terrestrial predators = 0.38, c.l. 0.09–0.67) than animals enclosed for the longer period (M = 0.09, c.l. 0–0.20), although the differences were only marginally significant (Z = 1.83, P = 0.066).

No differences were found between survival rates of females and males during the ten days following release after the confinement period (three nights: $Z = 0.67$, $P = 0.501$; 6 nights: $Z = 0.85$; $P = 0.393$).

Discussion

Although animals confined for a long period may exhibit a high mortality, as shown in this study, a confinement period of six nights did not increase rabbit mortality compared to others confined half time. Although assuming that we are comparing a short period (3 vs. 6 days), and/or the low number of animals tagged in our study, it is surprising that a slightly higher mortality was suffered during the shorter confinement period (12.9%). Therefore, at the end of the confinement period, an "underlying mortality" is acting, which seems to be due to handling related to the translocation process (i.e. capture, transport) and captivity itself (i.e. agonistic behavior) (Teixeira et al. 2007), since most of the deaths occurred inside the warren or with no signal of predation or disease, during the confinement period.

Once we opened the pen doors and rabbits were allowed to move freely in the study area, most deaths were due to terrestrial predators (mainly foxes). This is in accordance with most of the previous studies on rabbit translocation (e.g. Calvete et al. 1997; Moreno et al. 2004; Letty et al. 2008). However, in our case, as expected, a lower mortality occurred amongst animals held for the longer confinement period. Although we cannot assert the causes of the higher mortality, a shorter acclimation period is insufficient for an adequate settlement. Three nights was not time enough to make feel all rabbits released safe inside the warren. Perhaps some of them behaved as subordinate animals, or had no time enough to recover their former physical condition. In all the cases, these rabbits leaved quickly the warren (in our case the same day when allowed), and while searching for other place to settle, avoiding aggressions, or gathering better

condition, were killed. However, animals with six-acclimation nights adapted better to their release warren, and their mortality did not declined during the adaptation period. Moreover, in agreement with our hypothesis, three months later, most of the survivors (68%) remained in the warren where they were released (Rouco et al. 2008).

It is difficult to assess the optimal length of the confinement period comparing only two different lengths. However, if the relationship between nights confined and mortality associated were linear, it would be possible to estimate such regression with the mortalities obtained at the end of each period (0.46 and 0.80 for 3 and six-nights confinement periods), and the mortality obtained in a previous study conducted with no acclimation period (none nights of acclimation: 97%, Calvete et al. 1997). The result would be conclusive: mortality would become similar to the above mentioned underlying mortality due to confinement when rabbits are confined during 6.41 days (R^2= 0.995). Although we knowledge that the relationship of nights confined and mortality probably does not follows a linear curve, the low mortality during the adaptation period of the six-nights rabbits is clearly close of the optimal period, while three-days is still clearly far of it.

However, some final considerations regarding our results should be made. On the one hand, it is possible that other factors could also affect the optimum confinement period length. Our study was carried out in a low cover habitat, building artificial warrens which were basically the main refuge for rabbits, and it has been previously shown that cover may alter the dispersal distance (and therefore survival) of the released rabbits (Calvete and Estrada 2004). Finally, and more importantly, Letty et al. (2008) observed that relevant differences in survival of translocated rabbits could depend more on the quality of the habitat where released than on the length of the acclimation period. Therefore, gamekeepers and conservationists should take into account not only the

suitability of the habitat, but also the better acclimation when releasing rabbits in translocation programs.

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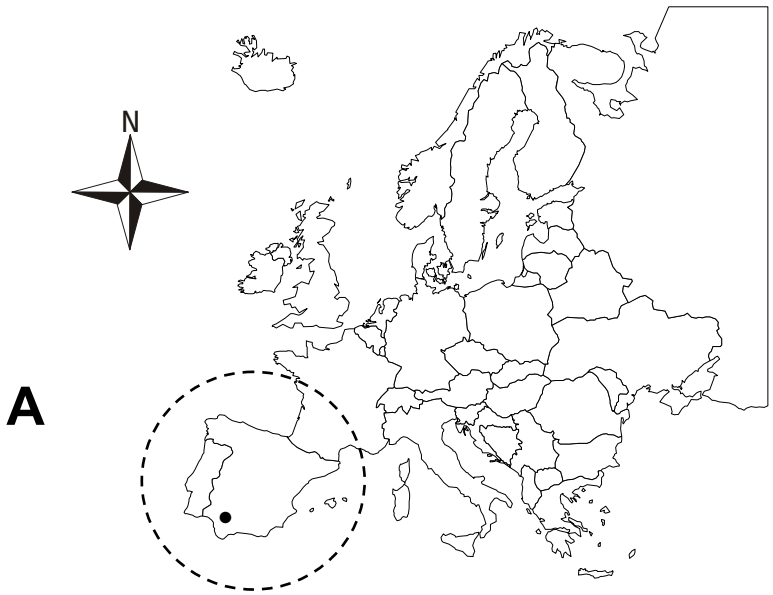
Figure Captions

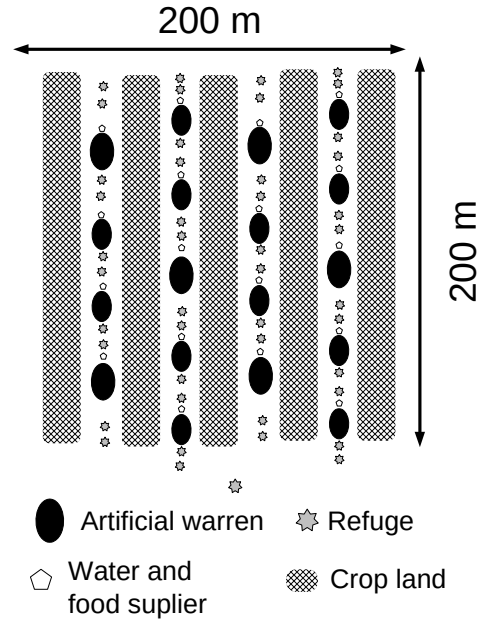
Figure 1. (A) Location of the Los Melonares area (●) on the Iberian Peninsula. (B) Structure of a translocation plot comprising artificial warrens, refuges, and water and food suppliers. (C) Detail of an artificial warren, location of the warren pen and doors.

Figure 2. Cumulative survival rates per day for each treatment group (three-nights of acclimation, and six-nights of acclimation) during the confinement period plus the following 10 days that compound the adaptation period.

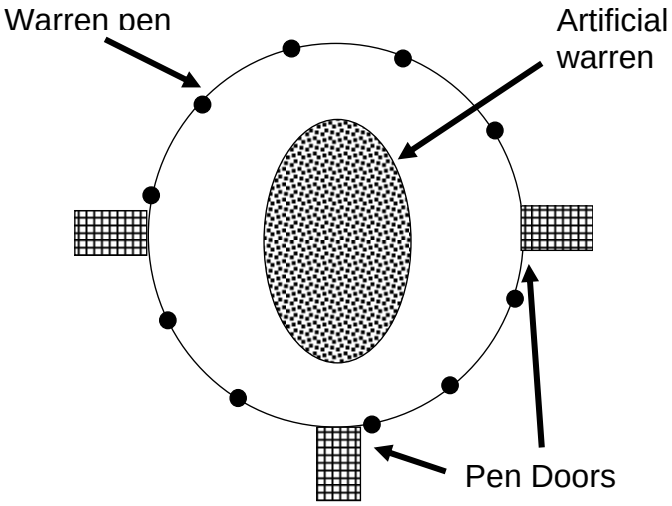
Figure 1


N

A

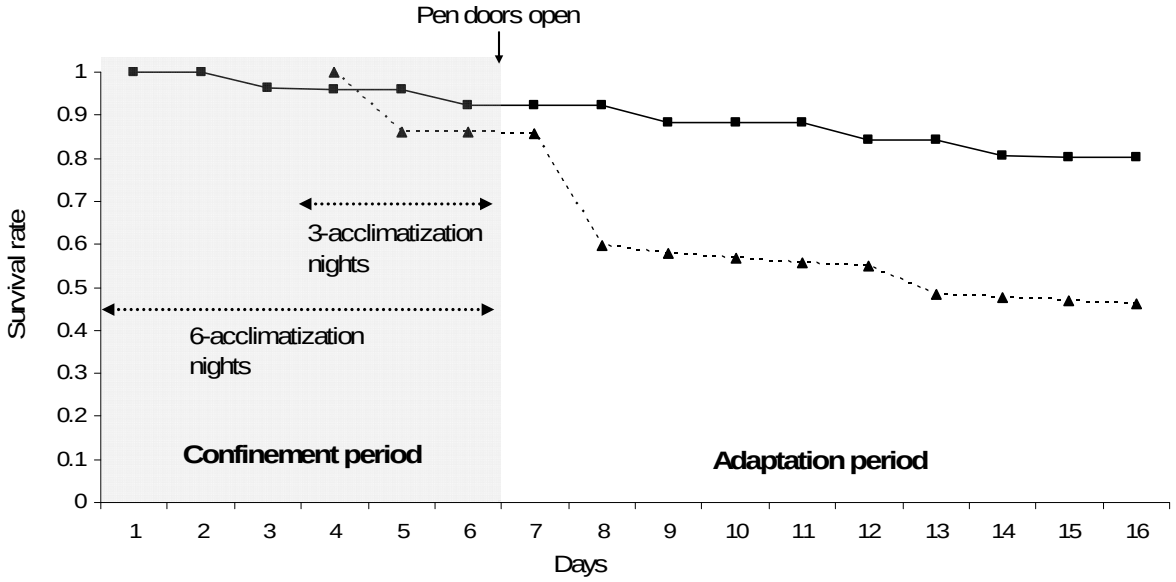

200 m
200 m
Artificial warren
Refuge
Water and food suplier
Crop land

B


Warren pen
Artificial warren
Pen Doors

C

Figure 2


Pen doors open
Survival rate
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3-acclimatization nights
6-acclimatization nights
Confinement period
Adaptation period
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Days