

## Translocation program 2023-2026 for the Dupont's lark *Chersophilus duponti*

(Project LIFE Connect Ricotí  
LIFE20 NAT/ES/000133)





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# ABSTRACT

Following IUCN Guidelines for Conservation Translocations, this document provides (1) background to the species and the translocation project, (2) a feasibility assessment including demographic and disease risks, and (3) a series of detailed protocols for management of the release and post-release phases including monitoring.

## Feasibility assessment

- Generally low risk of demographic impacts of harvest on source populations, assessed by population viability analysis.
- Modelling results suggest the translocation of approximately 8-10 individuals per year (6M/2H to 6M/4H) allows for chances of establishment while minimizing impacts on the donor population. Female survival and reproduction are the vital rates with the greatest effect on population viability and translocation success.
- Four extant subpopulations have been selected as sources, based on their current size and trend (ability to absorb harvest). Three destination sites were selected for release, based on habitat suitability, potential for connectivity, and legal and logistic constraints. Release sites will be subject to pre-release habitat restoration measures where necessary.
- Generally low expected disease risks, except for some classes of macroparasites. It is recommended that simple biosecurity protocols are implemented, and only healthy individuals are chosen for translocations following an in-situ health assessment.
- Uncertainty in post-release survival and dispersal by released individuals, particularly considering the difficulty of structuring releases by age. It is recommended that anchoring at release is assisted by carrying out translocations in the immediate pre-breeding period.

### Release protocols

- Capture sessions will take place immediately before the breeding season. Individuals will be caught and processed by a bird ringing specialist, which will include tagging with a VHF (very high frequency) transmitter and metal ring, where necessary, as well as a physical examination for all individuals.
- Those retained for translocation will be transported by car to the release site in individual cardboard boxes. At the destination, they will be health checked once more and released directly (hard release).
- Simultaneously, non-translocated individuals from the source population will be tagged with the same type of VHF transmitter (i.e., control individuals) to study differences between translocated and non-translocated birds.
- Post-release monitoring will focus on determining dispersal and survival of translocated and control individuals. Each released and control bird will carry a VHF transmitter and tracking will use an automatic receiving station (within the population of origin and destination) or handheld antennas (outside of both populations). The tracking station will be visited weekly to verify correct operation.



# CONTEXT

- 1.1 Species biology
- 1.2 Threats and conservation status
- 1.3 Conservation actions
  - 1.3.1 Legal framework of protection
  - 1.3.2 Past and current management



# 1. CONTEXT

## 1.1 Species biology

The Dupont's lark (*Chersophilus duponti*) is a small passerine bird belonging to the Alaudidae family, with a distribution restricted to Spain and northern Africa (Suárez, 2010). Biometric, genetic, and behavioural observations suggest that the populations in northern Africa belong to a different subspecies, although studies are currently being conducted to confirm this.

The Dupont's lark is a typical steppe bird known for its territorial behaviour (Pérez-Granados et al., 2016). It is considered a sedentary species, remaining in its breeding areas throughout the year (Suárez et al., 2006). However, dispersal movements or temporary migrations are known to occur during the harsh winter months, with individuals appearing in non-breeding areas (Suárez, 2010; García-Antón et al., 2021). Males defend their territories throughout the annual cycle (Suárez, 2010). On the Iberian Peninsula, the optimal habitat for the species is characterized by natural steppes with a predominance of small shrubs (20-40 cm) and low coverage of annual herbaceous plants (Garza & Suárez, 2010). It prefers areas with approximately 30% shrub coverage, a high percentage of bare ground (Tellería et al., 1988; Seoane et al., 2006; Suárez, 2010), and slopes not exceeding 10-15 degrees (Garza et al., 2003; Nogues-Bravo & Agirre, 2006; Seoane et al., 2006). The species tends to avoid hillsides, cultivated areas, wooded zones, pure herbaceous pastures, and areas with dense low shrub vegetation, such as gorse, rockrose, rosemary, or heather formations (Garza & Suárez, 2010). Altitude and climate do not seem to influence its distribution, as the species can be found from sea level (e.g., Almería) to areas located above 1,400 m (Garza & Suárez, 1990; Suárez et al., 2009b).

The species has cryptic plumage and an elusive nature, being extremely shy and reluctant to fly, even when humans are nearby (Tella et al., 2005; Vögeli et al., 2008). Most interactions with the species are auditory, and sightings are difficult to make. The breeding period for the Spanish population extends from late March to early July, with up to 3 breeding attempts (Pérez-Granados et al., 2017). Nests are usually built with a north orientation, on the ground next to a shrub, and not entirely covered (Pérez-Granados et al., 2017; Barrero et al., 2023). Clutch size ranges from 3 to 5 eggs (mean  $\pm$  standard error:  $3.47 \pm 1.15$ ), which are incubated for 12-13 days (Pérez-Granados et al., 2017). Being a nidifugous species, the chicks typically leave the nest at 8 days of age (Herranz et al., 1994; Suárez et al., 2009b; Garza & Suárez, 2010), although between 46% and 84% of nests are preyed upon before the chicks can leave the nest (Herranz et al., 1994; Suárez et al., 2009b; Suárez, 2010; Pérez-Granados et al., 2017). During the breeding period, the chicks are mainly fed with lepidopteran larvae, coleopterans, and arachnids (Herranz et al., 1993; Zurdo et al., 2023).

## 1.2 Threats and conservation status

The first national census of the Dupont's lark established a minimum population estimate for Spain, and thus for the Iberian Peninsula, of about 13,000 individuals (Garza & Suárez, 1988). Subsequently,

with the use of more precise and appropriate census methods for the species (Garza et al., 2003; Tella et al., 2005), the Spanish population was estimated to be around 3,100-4,000 males (Suárez, 2010). The latest estimation, with significantly greater sampling effort than previous censuses, yielded a minimum count of 3,828 males, mostly located in the communities of Aragón (1,614 males, 43.5% of the total) and Castilla-La Mancha (739 males, 19.3% of the total) (Traba et al., 2019). By province, Soria (1,091 males), Teruel (929 males), Zaragoza (663 males), and Guadalajara (646 males) account for 87% of the total counted males (Traba et al., 2019). It should be noted that the numbers from this last census were estimated with insufficient data for Aragón and Castilla y León (Traba et al., 2021). The male-biased sex ratio (Suárez et al., 2009a: 61%; Vögeli et al., 2007: 79%) drastically reduces the effective population size, and thus the number of females would be around 804-1,493. Therefore, assuming a margin of error in the estimation of the number of males, the effective population size of the Spanish (or European) Dupont's lark, updated in 2018, would be around 1,400-1,500 pairs and approximately 3,700-4,000 males (Traba et al., 2019). These data have recently been reviewed and suggest a significant decline of around 25% (Reverter et al., in preparation).

Currently, the species only persists in four of the six regions mentioned in the I National Census: Sistema Ibérico, Ebro Valley, Southern Meseta, and Southeast (García-Antón et al., 2019), having become extinct in the Northern Meseta and Zamora regions (Traba & Garza, 2021). The occupied area in Spain barely exceeds 1,010 km<sup>2</sup>, distributed in a fragmented manner among 23 populations and 100 subpopulations (Traba et al., 2019), with even less suitable area for supporting breeding populations (698 km<sup>2</sup> according to García-Antón et al., 2019), although the latest revision again suggests a decrease in the distribution area (Reverter et al., in preparation).

The most important or 'core' subpopulations (Sistema Ibérico and Ebro Valley), although declining, still present a relatively good conservation status; however, they have also experienced processes of demographic contraction and range reduction, with extinctions or severe local declines (Reverter et al., in preparation). The most peripheral or marginal subpopulations are at an extremely high risk of extinction (García-Antón et al., 2021). The main threats facing the species are habitat loss and alteration, favouring isolation and the fragmentation of their populations (Iñigo et al., 2008; Traba et al., 2019). The only habitat used by the species (natural steppes) is in decline throughout Spain, with a highly fragmented and dispersed distribution. Recent studies suggest metapopulation behaviour (García-Antón et al., 2021), with relatively independent subpopulations presenting their own probabilities of extinction and dispersal movements connecting them. In fact, recent studies indicate a continued low genetic structuring of the Iberian population (Bustillo-de la Rosa et al., 2022), suggesting some gene flow between certain subpopulations. In this sense, there is evidence of recolonization events in previously extinct populations, such as Alfés, Lleida (Bota et al., 2016).

Habitat reduction is mainly due to the conversion of natural steppes into cultivated lands, afforestation, or the installation of infrastructures such as wind or photovoltaic power plants (Suárez, 2010; Traba et al., 2019). It is also a result of vegetation succession towards dense shrub or forest structures, due to the abandonment of extensive livestock grazing (Martínez-Valderrama et al., 2021; Traba & Pérez-Granados, 2022). Recent studies (Gómez-Catasús et al., 2019; Gómez-Catasús et al., 2023) have

highlighted the close link between sheep farming and the Dupont's lark, as extensive sheep grazing plays a key role in maintaining a suitable vegetation structure for the species, while also providing dung that favours the presence of beetles, which are a fundamental part of its diet (Zurdo et al., 2023). The abandonment of traditional extensive grazing and land use changes, therefore, pose a serious threat to the remaining populations.

According to data collected from 92 populations in Spain, the Dupont's lark experienced an annual decrease rate of 3.9% and an overall decrease of 41.4% during the period 2004-2015 (Gómez-Catasús et al., 2018). This result aligns with previous findings in Spain, indicating a decline of 31.5% over 16 years ( $n = 34$  populations; Tella et al., 2005) and up to 70% over 12.5 years ( $n = 33$  populations; Pérez-Granados & López-Iborra, 2014). Regarding trends by regions, Andalucía and Castilla y León appear to show a drastic decrease in numbers up to 2015 (annual decrease rate exceeding 5%), while Aragón, Castilla-La Mancha, Catalonia, Valencian Community, Navarra, and Murcia exhibit uncertain trends (Gómez-Catasús et al., 2018).

The situation since 2015 shows signs of deterioration. In important areas for the species, such as the Special Protection Area (SPA) of Altos de Barahona (Soria), change rates of -36.5% between 2017 and 2020 have been detected (personal data). More recently, obtained preliminary results for 2021 in Altos de Barahona and Páramo de Layna (Soria), Alfés (Catalonia), Rincón de Ademuz (Valencia), and localities in Guadalajara show even worse outcomes, with decline rates between 2020 and 2021 ranging from -30% to -60% (Pérez-Granados et al., 2023). These sharp declines could be attributed to extreme weather events during the winter of 2020 (snowstorm 'Filomena') that affected already depleted populations. If these data are confirmed, the Iberian population could have been reduced in a single year to an effective population size of 600-1,500 pairs, increasing the fragmentation and isolation of the remaining populations (Reverter et al., in preparation). As for the distribution area, there has been a significant reduction process since the late 1980s. Considering the areas for which accurate information on historical distribution is available, a reduction of 44.1% has been observed between the periods before and after the year 2000 (García-Antón et al., 2019).

### 1.3 Conservation actions

#### 1.3.1 Legal framework of protection

The Dupont's lark is one of the most threatened European passerines, classified as 'Endangered' in the Red Book of Birds of Spain (Traba et al., 2021) and in the latest assessment of bird conservation in Spain conducted by SEO/Birdlife (2021). Likewise, in the Spanish Catalog of Endangered Species (Real Decreto 139/2011, de 4 de febrero), it is classified as 'Endangered' (BOE-A-2023-8751).

At the European level, it is listed in Annex I of the Birds Directive (Dir. 79/409/EEC) as a species subject to conservation measures and is categorized as 'Vulnerable' in the European Red List (2021). Spain holds the greatest responsibility for the conservation of this species as it only nests within its territory in Europe (Traba et al., 2019). On a global scale, the species is classified as 'Vulnerable', both

in the European population and in the total of its worldwide populations (European and African) (IUCN, 2022).

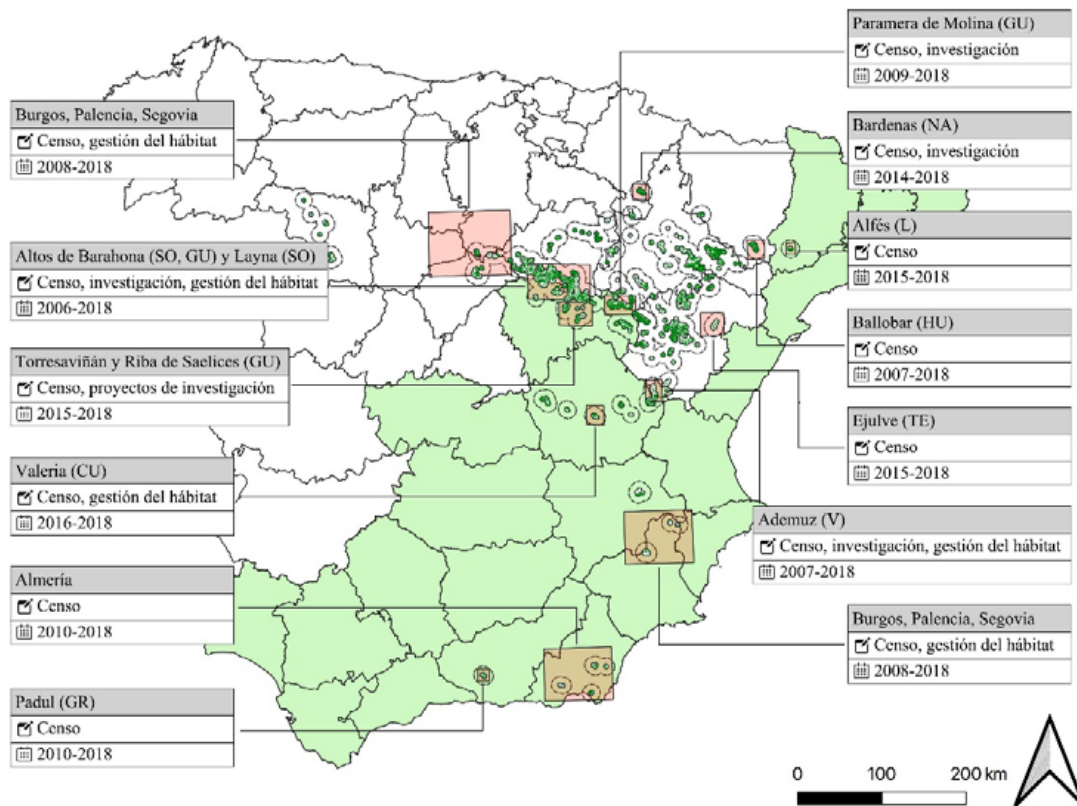
### 1.3.2 Past and current management

The Dupont's lark has attracted the attention of numerous studies, and there is currently a consensus within the scientific community regarding the conservation actions that should be implemented to halt the ongoing decline of the species. However, there is a marked disparity between the actions proposed by scientists and those frequently implemented by managers. Scientists' proposals have mainly focused on regulation/legislation and/or management interventions. According to Pérez-Granados and López-Iborra (2022), these proposals represent 45% and 42%, respectively, of the total proposed conservation actions from the scientific community, while managers predominantly carry out monitoring and research actions for the species, accounting for 50% of the total actions. In this context, management actions, crucial for the conservation of the species, traditionally only represent 20% of the total interventions conducted (Pérez-Granados & López-Iborra, 2022). It is significant that out of the 20 areas where the monitoring of Dupont's lark populations was conducted between 2007 and 2018 (see below), only a few implemented habitat management actions: Ademuz (Valencia), promoted by the regional administration; Altos de Barahona/Layna (Soria), by the LIFE Ricotí project; in Andalusia during 2021 and 2022, projects were undertaken in Cabo de Gata (Almería), where the species currently persists, and in Dehesa Guadix (Granada), where it existed in the past. Other actions are currently in their early stages (Castilla-La Mancha, Castilla y León, Catalonia; LIFE Connect Ricotí).

Understanding why managers implement certain conservation actions is useful in bridging the gap between research and conservation strategies. Because of the precarious conservation status of the Dupont's lark, regional authorities are obligated to provide periodic updates on population estimates. This may explain managers' interest in implementing research or monitoring interventions (Pérez-Granados & López-Iborra, 2022). On the other hand, management actions often have higher economic costs compared to basic monitoring and research, making their execution frequently dependent on budget availability (Gibbons et al., 2011; Pérez-Granados & López-Iborra, 2022). These management interventions are usually costly and logistically challenging to implement (Bertuol-Garcia et al., 2018; Walsh et al., 2019) as they require specialized personnel and equipment, as well as monitoring of the effectiveness of the conservation measure implemented (Shea et al., 2002; Scott et al., 2010). In this regard, projects focused on the application of management/conservation measures, as well as their evaluation, are currently essential for improving the flow and application of knowledge.

During the period 2007-2018, projects specifically focused on the Dupont's lark (*Chersophilus duponti*) were implemented in 20 zones within its distribution area in Spain. Monitoring studies were carried out in all Autonomous Communities (CCAA) (Figure 1.1), mostly promoted and directed by the respective CCAA, although there was also involvement from private entities (e.g., Granada, Burgos, Palencia, Segovia, Valencia), local initiatives (Navarra), or European conservation projects (Soria, LIFE Ricotí project). The knowledge derived from these projects has varied across different CCAA, reflec-

ting the uneven coverage of the species' distribution area in each project.



**Figure 1.1.** Monitoring, research, and management projects of Dupont's lark populations conducted during the period 2007-2018. Autonomous Communities (CCAA) with up-to-date information on the status of all their populations are indicated. CCAA with updated information are highlighted in green (Andalucía, Castilla La Mancha, Cataluña, Murcia, Valencia) although there is an error in the data referring to Murcia in the original source (Traba et al. 2019).

In peripheral populations, such as those in Andalucía, Cataluña, Murcia, and Valencia, the available information on population sizes and distribution is quite comprehensive as these Autonomous Communities (CCAA) have very few populations, and annual monitoring is carried out by the regional authorities. Another peripheral population is located in Navarra, but the available information is restricted to the population in Bardenas, where a local institution has been promoting various studies and periodic censuses of the species. In contrast, in the CCAA that host larger populations, the time series information is not as complete, although recent updates on population sizes and distribution have been made, for example, in Castilla-La Mancha (Garza et al., 2018; Traba & Garza, 2018) and Castilla y León (Traba & Garza, 2020), owing to the projects carried out in some of the most important Iberian populations of the species (LIFE Ricotí project). All these projects include, at a minimum, a census of the number of males, obtained during the early morning and adjusted to the peak singing activity of the species, although there are some differences in the census methodology applied between regions. However, the current situation of the populations in Aragón, the autonomous community that


probably hosts the largest number of individuals in Spain, is still unknown.

The results of all these projects are contributing to the establishment of management and conservation guidelines for the species based on scientific studies (Traba et al., 2019). These projects have generated information that has helped to identify areas of special protection for the species, recommend agricultural practices compatible with conservation, as well as expand basic knowledge about the species' biology and ecology. The Iberian and North African distribution areas have been defined and updated, as well as population sizes and their genetic connectivity. Additionally, research has delved into the species' spatial ecology and the impact of human activities (e.g., renewable energies, fires, agriculture).

Regarding direct conservation actions, the Generalitat Valenciana has carried out habitat restoration actions in the populations of Rincón de Ademúz. Specifically, in 2015 and 2018, clearings were performed to reduce shrub vegetation cover by 30%, and large pines (>5 m) were removed in 55 ha across two Dupont's lark populations. In parallel, extensive grazing and the artificial addition of sheep dung were promoted (Saez-Gomez et al., 2021).

On the other hand, during the course of the LIFE Ricotí project (LIFE15-NAT/ES/000802; 2016-2021), 326 ha were successfully restored, allowing for the establishment of 39 new territories of the Dupont's lark (data obtained in 2019). Over 3,000 ha were incorporated into the Land Stewardship Program, which addressed the promotion of extensive grazing. Moreover, the Dron-Ricotí project (BBVA Foundation, 2016-2019) identified the fundamental relationships between sheep grazing, food availability, and the presence of the Dupont's lark (among other steppe birds). In this regard, the Basic Scientific Guidelines for the National Conservation Strategy of the species have established priorities and lines of action for its conservation (see Traba et al., 2019). In these guidelines, the **maintenance and improvement of connectivity** in the Iberian metapopulation is a priority to be achieved through i) **increasing high-quality habitat** in key areas (structural connectivity), and ii) **reinforcing/rescuing populations** at high risk of extinction and/or critical for the connection of the Iberian metapopulation (assisted connectivity).

Currently, the TEG-UAM is coordinating the LIFE Connect Ricotí project (LIFE20 NAT/ES/000133; 2021-2026), which aims to improve the conservation status of the Iberian metapopulation of Dupont's lark by increasing its structural and functional connectivity through actions on several key subpopulations. This is being addressed with two main lines of action: i) increasing high-quality habitat for the species (structural connectivity); and ii) recovering or reinforcing populations at high risk of extinction with wild individuals from donor locations (assisted connectivity). The increase in high-quality habitat is achieved through shrub clearance and selective tree removal, as well as the promotion of extensive sheep grazing. The increase in connectivity will reduce the risk of local extinction in the target subpopulations (Hanski, 1998), without affecting the survival of donor subpopulations and the entire metapopulation. The Dupont's lark is a paradigmatic example of population decline, as the Iberian population still comprises a few thousand individuals but shows a clearly negative trend, making it necessary to take action before the population collapses.

A tall, silver antenna tower stands on a grassy hill. The tower has a complex structure of horizontal arms with various antennas attached. It is supported by a tripod base and several blue guy lines. In the background, a group of people in winter clothing are standing on the hill, looking towards the tower. The sky is overcast and grey.

# 2 THE TRANSLOCATION PROGRAM

2.1 Motivation

2.2 Objectives

## 2. THE TRANSLOCATION PROGRAM

### 2.1 Motivation

Conservation strategies for threatened species increasingly include translocation programs for reintroduction, reinforcement, or recovery of their populations (Parker, 2008). These programs serve as an effective tool for restoring ecosystem processes and population connectivity (Benayas et al., 2009; Seddon et al., 2014). Assisted connectivity can be achieved through reintroduction (if necessary, followed by reinforcement) in locations where extinction has occurred or via reinforcement in populations that still persist but face a high risk of extinction and/or in key areas contributing to overall connectivity, thus increasing metapopulation stability.

The current situation of the species in Spain aligns with the IUCN recommendations for initiating translocations of wild individuals (IUCN, 2013):

- Translocation does not extend beyond the known historical distribution range of the species and therefore poses minimal risks of unintended effects on the ecosystem of the release area, including risks of disease, predation, competition, and hybridization (Blackburn et al., 2014).
- Genetic similarity between donor and recipient subpopulations is sufficiently high to avoid outbreeding depression, as demonstrated by the results of the LIFE Ricotí project (Bustillo-de la Rosa et al., 2022). Conversely, translocating individuals can mitigate the genetic depression of the species, which occurred during the extinction process (Méndez et al., 2011; Bustillo-de la Rosa et al., 2022).
- Concurrently with translocations, the planned habitat management measures in the target populations have been successfully tested (LIFE Ricotí: LIFE15-NAT/ES/000802; 2016-2021) and appear adequate to eliminate or sufficiently reduce the threats that caused the previous extinction (IUCN, 2013). Both strategies (habitat restoration and improvement, and translocation) could contribute to halting the decline of the species and establishing a framework for future conservation efforts at the national level.

### 2.2 Objectives

The main objective of the LIFE Connect Ricotí project is to improve the conservation status of the Iberian metapopulation of the Dupont's lark by increasing its structural and functional connectivity, targeting several key subpopulations. This will be addressed through two major lines of action:

- a) Increase high-quality habitat for the species (structural connectivity) in three Spanish regions (Catalonia, Castilla-La Mancha, and Castilla y León). This will involve expanding a unique and fragmented natural habitat of high conservation interest that supports the Dupont's lark and other relevant bird species. While indirectly relevant to this translocation program, this objective will not be discussed further.



b) Enhance connectivity by reinforcing recently extinct or critically endangered subpopulations using wild animals from donor locations. Connecting the source and target sites and providing population reinforcement to the latter will delay the local extinction of peripheral subpopulations (Hanski, 1998), without affecting the persistence of donor subpopulations or the entire metapopulation. The Iberian population of the Dupont's lark currently consists of a few thousand individuals but exhibits a clearly negative trend, making it crucial to act before a population collapse occurs. In the short term, increasing the distribution area avoids the risk of synchronous stochastic fluctuations in subpopulations (Pérez-Granados et al., 2023) and extends the expected time until extinction (see population viability analysis below). In the long term, it enhances variability in geographical differences in gene frequencies, thereby increasing long-term survival capacity.

For this second objective, the specific goals of this translocation program are as follows:

1. Evaluate the translocation of wild animals as a key factor in reinforcing/rescuing areas where the species is already extinct or nearly extinct with extremely negative population trends, and where natural recolonization is highly unlikely (assisted connectivity).
2. Connect critical areas for population connectivity (i.e., increase metapopulation stability).

The individuals will be sourced from healthy populations in Castilla-La Mancha.



# FEASIBILITY STUDY

## 3.1 Demographic study

### 3.1.1 Methodology

### 3.1.2 Results

## 3.2 Populations and source/destination areas

### 3.2.1 Selection of source populations

### 3.2.2 Selection and preparation of destination areas

## 3.3 Disease risk analysis

### 3.3.1 Methodology

### 3.3.2 Results and recommendations

## 3.4 Risk to animal welfare

## 3.5 Adaptative management and emergency plan

## 3. FEASIBILITY STUDY

### 3.1 Demographic study

The demographic feasibility of the translocation program was assessed through a population viability analysis (PVA) as suggested by Traba et al. (2019). In brief, the results show that reinforcing the three recipient populations with a small number of individuals (6 males, 2-4 females) over 3 years significantly prolongs the viability of these populations, while the removal of individuals has no effect on the donor populations. A summary of the methods and results of this PVA is presented here, but a complete description can be found in Appendix 3.2.

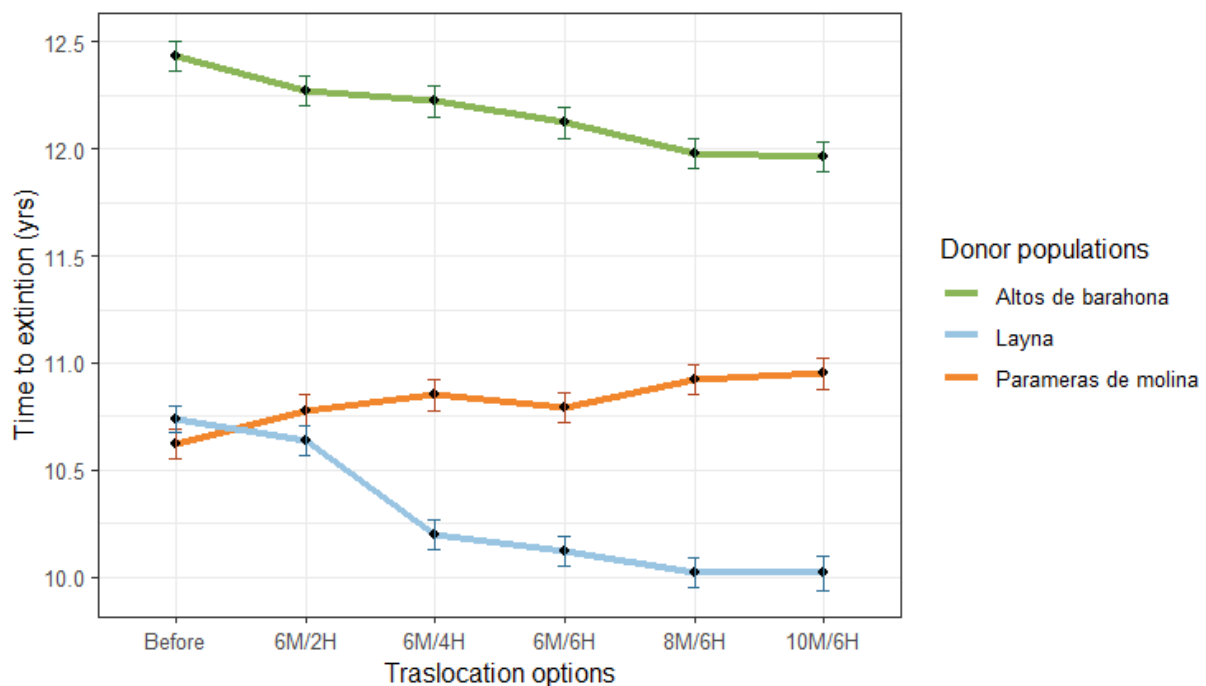
#### 3.1.1 Methodology

The PVA was conducted using the stochastic simulation program VORTEX 10.5.6 (Lacy & Pollak, 2014), running individual-based models with 1,000 iterations to encompass demographic, environmental, and genetic stochasticity. To assess the medium- to long-term survival of the metapopulation, the models were projected for 20 years, as established by IUCN criteria. All PVAs were designed at the subpopulation level, based initially on the structure of the Iberian metapopulation (García-Antón & Traba, 2021), but exclusively using subpopulations wholly or partially included in Castilla-La Mancha, the region where the translocation program is intended to be implemented. In each iteration, a subpopulation was considered extinct when at least one of the two sexes reached extinction. The main parameter was the mean time to metapopulation and subpopulation extinction.

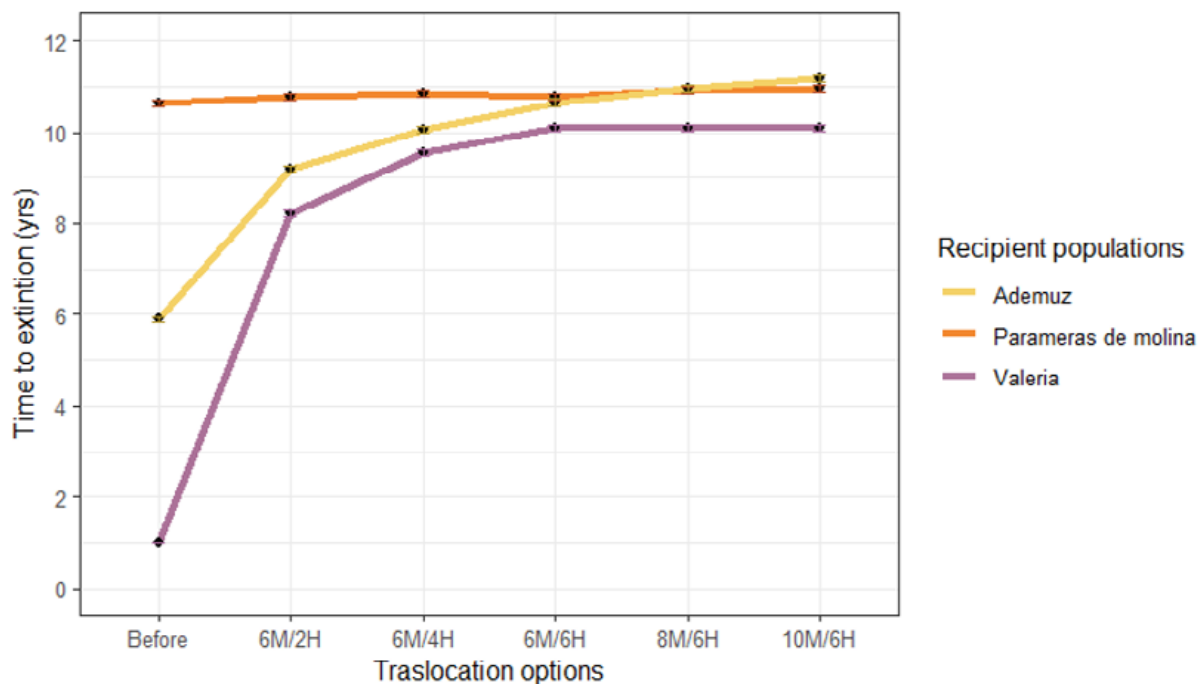
The PVA was conducted in two steps. Firstly, a baseline model was constructed, considering the most plausible value for each population parameter in relation to the available current information, and then a sensitivity analysis was performed on the baseline model (without translocations) to determine the most relevant demographic parameters on metapopulation viability and to assess the effect of uncertainty and variability on the reference projections. Secondly, the translocation process was simulated to evaluate its effect on both donor and recipient populations. In this regard, the baseline model was modified with different translocation scenarios (varying numbers of males and females) and with different levels of immediate post-translocation survival/settlement (to represent the probability of mortality and/or dispersal away from the release site). These scenarios considered the increase in available habitat resulting from restoration actions. Finally, the effects of all these scenarios on the mean time to extinction of both donor and translocated subpopulations were evaluated.

### 3.1.2 Results

The simulation of the translocation process showed that the removal of individuals from the donor populations in Parameras de Molina (Molina de Aragón), Altos de Barahona, or Layna did not worsen, or at least not significantly, the mean time to extinction of those populations (Figure 3.1). On the contrary, the time to extinction significantly increased in the recipient populations if at least 8-10 individuals (6M/2-4F) were released (Figure 3.2). Immediate dispersal or mortality after release could reduce this mean time to extinction by up to 50% (Appendix 3.2). Based on these results, a translocation of 8-10 individuals per year, with an equal proportion of sexes, when possible, provides a high probability of success with low risk for the selected source populations (Section 3.2). Post-release survival (including dispersal) is a key factor for success and presents uncertainty, particularly considering the challenges in determining the age of released individuals (Section 4.1). Therefore, in the event of insufficient survival, it may be possible to modify the protocol to implement additional measures that minimize dispersal and promote site fidelity, followed by specific monitoring (Section 3.5).



**Figure 3.1.** On the x-axis, different translocation combinations are indicated, according to the number of translocated males and females. The y-axis shows the mean time to extinction for each of the donor subpopulations (over 1,000 iterations).



**Figure 3.2.** On the x-axis, different translocation combinations are indicated, according to the number of translocated males and females. The y-axis shows the mean time to extinction for each of the recipient subpopulations (over 1,000 iterations).

## 3.2 Populations and source/destination areas

### 3.2.1 Selection of source populations

The selection of 'source' or origin populations for these translocations (Table 3.1, Figure 3.3) was conducted through PVA (Section 3.1). The chosen subpopulations have sufficient population sizes (190, 92, and 631 males in Anguita, Páramos de Molina, and Atienza, respectively; years 2021-22, according to data until 2021; García-Antón & Traba, 2021) to provide the required individuals for the translocation program without compromising their survival (Section 3.1). Annual censuses will be conducted in the donor populations to obtain precise estimates of the population size before and after translocations.

### 3.2.2 Selection and preparation of destination areas

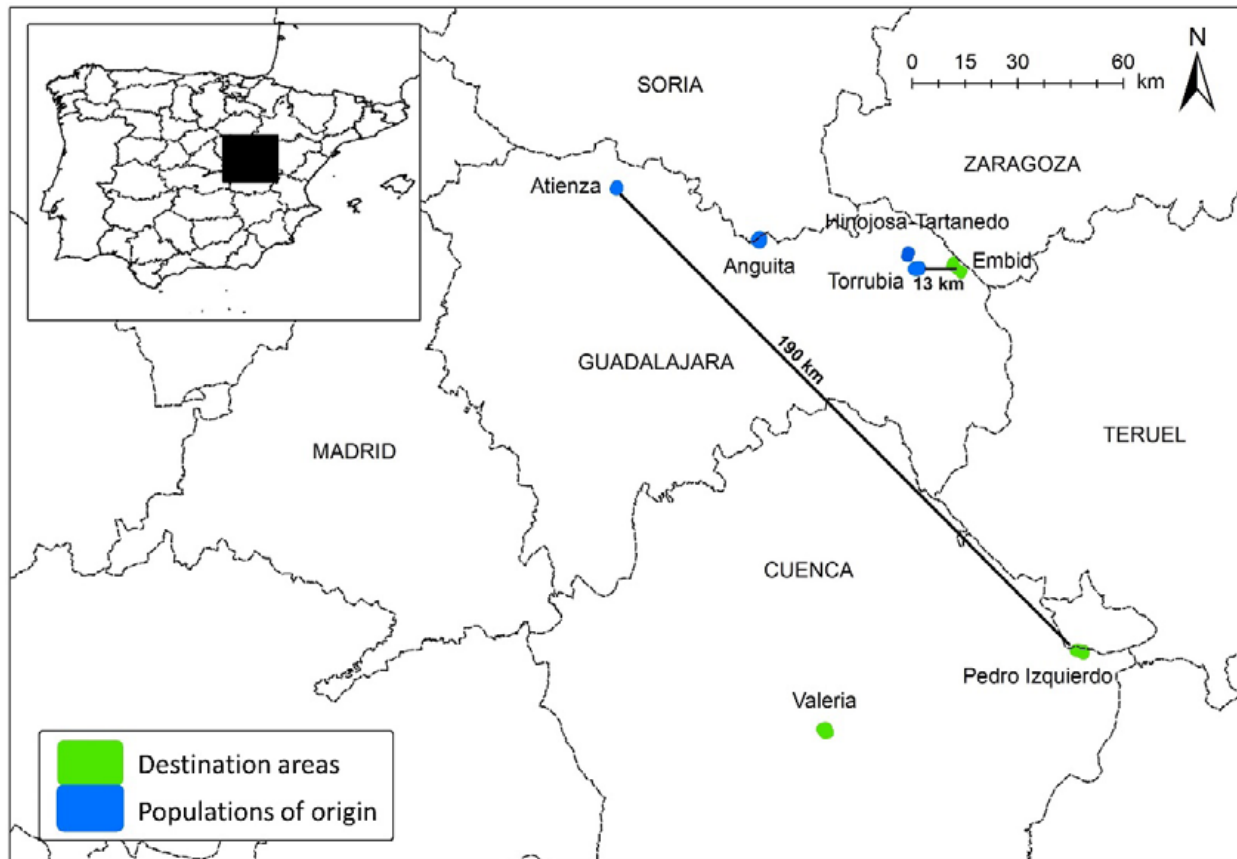
The selection of release sites (Table 3.1) was based on the following criteria: (1) previous presence of the Dupont's lark (Traba et al., 2021); (2) habitat requirements (Traba et al., 2021); (3) connectivity (García-Antón et al., 2021); and (4) logistical and administrative feasibility. Similar to the source locations, pre- and post-release censuses of the birds will be conducted in the destination areas. The release sites must have suitable habitat for the species (Section 1.1). The selection of release areas considered the available habitat area, with priority given to zones with larger suitable habitat areas.

Connectivity of the release area and population size were positively considered in the selection of areas. Thus, populations with higher connectivity values were prioritized over those with lower significance according to the connectivity model of the Iberian Dupont's lark metapopulation (García-Antón et al., 2021), positively promoting areas close to those with larger populations and/or those that are particularly important for the overall connectivity of the metapopulation. The distance between source and destination populations ranges from 190 to 13 km (Figure 3.3), allowing for the assessment of the success of translocations at two spatial scales, short and medium distance. Population size was also considered, with areas close to occupied territories with a larger number of Dupont's lark individuals being prioritized over areas with smaller population sizes.

Lastly, within the areas selected on the basis of the above criteria, priority will be given to those where translocations are most feasible, both logistically (e.g., accessibility) and administratively (agreements with landowners, obtaining permits). If necessary, before conducting translocations, a series of direct habitat management measures will be implemented in the selected areas to improve their quality and quantity and enhance the success of future translocations (Section 1.2).

**Table 3.1.** Selected subpopulations for the translocation of Dupont's lark individuals using the Iberian metapopulation structure proposed by García-Antón et al. (2021). The source/destination of each subpopulation is indicated, as well as the objective of the translocation.

Province	Municipality	Subpopulation	Action C3	Objective
Cuenca	Pedro Izquierdo	Ademuz	Destination	Stepping stone
Cuenca	Valeria	Valeria	Destination	Rescue
Guadalajara	Embid	Parameras de Molina	Destination	Reinforcement
Guadalajara	Torrubia	Parameras de Molina	Source	
Guadalajara	Tartanedo-Hinojosa	Paramers de Molina	Source	
Guadalajara	Anguita	Layna	Source	
Guadalajara	Atienza	Altos de Barahona	Source	



**Figure 3.3.** Geographical location of the selected source and destination populations for the translocations over three years within the framework of the LIFE Connect Ricotí project (LIFE20 NAT/ES/000133).

### 3.3 Disease risk analysis

#### 3.3.1 Methodology

The International Union for Conservation of Nature (IUCN) recommends conducting a disease risk analysis (DRA) and intensive monitoring of all translocated animals (IUCN, 2013). A DRA is a structured, evidence-based process that can assist in decision-making and determining the potential impact of infectious and non-infectious diseases on ecosystems, wildlife, domestic animals, and humans (Jakob-Hoff et al., 2014). To assess the disease risk of the Dupont's lark translocation program, the procedures described by Jakob-Hoff et al. (2014) and the methodology published by Sainsbury and Vaughan-Higgins (2012), updated by Bobadilla Suarez et al. (2017) and Rideout et al. (2017), were followed. While a summary of the methods and results of this DRA is presented here, the reader is referred to Appendix 3.3 for a complete description.

In the first step, published literature and unpublished veterinary records describing diseases that may affect passerine species and other Iberian birds were reviewed. The information was used to create a list of hazards that may be relevant in the translocation of the Dupont's lark in the central Ibe-

rian plateau. Next, expert veterinarians from the Wildlife Conservation Medicine (WildCoM) research group at the Autonomous University of Barcelona reviewed the preliminary hazard list with informative notes and made appropriate corrections based on their knowledge and personal experience. The obtained list of identified hazards is presented in Table 3.2. On the basis of this preliminary list of 37 identified hazards, the experts prioritized the hazards according to the probability of exposure and the magnitude of consequences in the case of exposure. For each hazard, exposure probability and consequences were assessed for the three at-risk populations in four categories: Negligible, Low, Medium, High. For each of the hazards of special concern to the advisory group, further risk assessments were performed, on the basis of exposure and consequence evaluations, in increasing risk categories from 0 to 3 (Table 3.3).

**Table 3.2.** List of identified hazards for the proposed translocation of the Dupont's lark (*Chersophilus duponti*). The colours indicate the detection of the hazard in different bird taxa or the absence of the hazard in Spain.

Viruses	Bacterias	Protozoa	Fungi	Ectoparasites	Endoparasites	Non-infectious
Adenovirus	Campylobacter sp.	Intestinal coccidia	Aspergillus fumigatus	Feather mites	Intestinal cestodes	Predation
Avipoxvirus	Chlamydophila psittaci	Hermoparasites	Candida sp.	Ticks	Intestinal nematodes	Neoplasia
Circovirus	Clostridium botulinum	Trichomonas sp.		Feather lice		Toxins
Flavirus (West Nile Virus)	Clostridium perfringens					Traumatism
Herpesvirus	Erysipelothrix rhusiopathiae					Dehydration at capture and transport
Influenza	Escherichia coli					Lesions derived from radio transmitter placement
Newcastle	Mycobacterium avium					
Polyomavirus	Mycoplasma sp.					
	Pasteurella multocida					
	Salmonella sp.					
	Klebsiella spp.					
	Suttonella ornithocola					
	Yersinia sp.					

In <i>Chersophilus duponti</i>	In Alaudidae	In passerines	In other bird taxa	Out of Spain
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**Table 3.3.** Classification of identified hazards for the proposed translocation of the Dupont's lark (*Chersophilus duponti*) according to the experts.

HAZARDS	TRANSLOCATED LARKS		FAUNA		HUMANS	
	Probability	Consequences	Probability	Consequences	Probability	Consequences
<b>INFECTIOUS</b>						
Adenovirus	1	0	1	0	0	0
Avipoxvirus	1	1	0	1	0	0
Circovirus	1	0	1	0	0	0
Flavivirus	1	0	0	0	0	1
Herpesvirus	1	1	1	1	0	0
Influenza	1	1	1	1	0	1
Newcastle	1	0	1	0	0	0
Polyomavirus	0	1	0	1	0	0
Campylobacter sp.	2	0	1	0	1	0
Chlamydomydia psittaci	1	1	1	1	1	1
Clostridium botulinum	0	1	0	1	0	1
Clostridium perfringens	1	1	1	1	0	0
Erysipelothrix rhusiopathiae	0	0	0	0	0	0
Escherichia coli	3	1	1	1	1	1
Mycobacterium avium	1	1	1	0	0	0
Mycoplasma sp	1	1	1	1	0	0
Pasteurella multocida	0	1	0	1	0	1
Salmonella sp.	1	1	1	1	1	1
Klebsiella sp.	1	1	1	0	0	0
Suttonella ornithocola	0	0	0	0	0	0
Yersinia sp.	1	1	1	1	1	1
Coccidiosis intestinales	2	2	1	1	0	0
Hemoparasitos	2	1	1	1	0	0
Trichomonas sp.	0	0	0	0	0	0
Aspergillus fumigatus	2	1	0	0	0	0
Candida sp.	1	1	1	0	1	1
Ectoparasites	3	1	1	1	1	0
Endoparasites	2	0	1	0	0	0
<b>NON-INFECTIOUS</b>						
Predation	1	2	N/A	N/A	N/A	N/A
Neoplasia	0	0	N/A	N/A	N/A	N/A
Toxins	0	0	N/A	N/A	N/A	N/A
Traumatism	2	1	N/A	N/A	N/A	N/A
Dehydration at capture and transport	2	1	N/A	N/A	N/A	N/A
Lesions derived from radio transmitter placement	1	1	N/A	N/A	N/A	N/A

0 = Negligible, insignificant; 1 = Low, morbidity or mortality at the individual level with no consequences for the population; 2 = Medium, may cause transient population issues; 3 = High, risk of population decline or extinction; NA = not applicable.

### 3.3.2 Results and recommendations

The following hazards were selected for a detailed risk assessment:

- Ectoparasites
- Coccidia
- Hemoparasites
- *Aspergillus fumigatus*
- Enterobacteria

Particular recommendations include:

- Conduct a pre-release physical examination, with sample collection for pathogen identification (Section 4.1.5).
- Take simple safety measures such as using new gloves for each handled individual and proper disinfection of materials.
- Transport individuals individually in adequately ventilated boxes (Section 4.2.2).

### 3.4 Risks to animal welfare

In addition to a disease risk analysis, IUCN guidelines recommend incorporating considerations of planned activities that may affect the welfare of wild animals in translocation plans (IUCN, 2013). Identifying risks to animal welfare has the dual objective of maximizing the animals' well-being during translocation and maximizing the success of translocation from a population perspective (Harrington et al., 2022). Therefore, in the translocation program for the Dupont's lark, a list of potential risks to animal welfare during the current translocation has been evaluated, following the 'Five Domains' model (Harvey et al., 2020). This model allows for identifying compromises in four physical/functional domains (nutrition, environment, health, and behaviour) and a mental domain that reflects the animal's affective experiences. While a summary of the methods and results of this animal welfare analysis is presented here, a complete description can be found in Appendix 3.4.

The risks have been classified into 1) risks during capture, 2) risks during transportation, and 3) risks after release (Harrington et al., 2022). For the mitigation of each risk, corrective measures are recommended and more extensively detailed in other sections of this document (Sections 3.3 and 4.1). The present translocation program includes an emergency or contingency plan in case these risks prove to be unacceptable (Section 3.5).

During the capture phase, there is considered to be a **high probability** of distress, fear, or anxiety due to capture and handling, although the likelihood of injury or death is moderate or low. To mitigate this risk, procedures will always be carried out by experienced professionals, and their duration will be minimized as much as possible (Section 4.1).

During the transportation phase, there is considered to be a **high probability** of distress, fear, or anxiety due to transportation, and a high probability of thermal discomfort, ventilation issues, or vehicle movement-related discomfort during transport. To mitigate this risk, transportation will be con-

ducted in individual cardboard boxes with perforations on the sides. Additionally, the transportation phase should not exceed a maximum of 6 hours (including capture, handling, displacement, tagging, and animal release). The release of excessively stressed or injured animals will be avoided through a second clinical examination at the destination site (Section 4.2).

During and after the release phase, there is considered to be a **high probability** of distress, fear, or anxiety due to marking and tracking methods. To mitigate this risk, marking methods will be exclusively carried out by professionals with prior experience (Section 5.2). The transmitters will never exceed 3% of the individual's body weight, as this has been shown to significantly reduce the incidence of behavioural and health issues (Geen et al., 2019). All released animals will be monitored, providing information on behaviour, reproduction, and survival (Section 5).

In summary, the proposed Dupont's lark translocation program carries a **moderate risk to animal welfare**. The exact magnitude and impacts of these risks are unknown as there are no previous occurrences of translocating this species. The monitoring protocol described in this document (Section 5) is designed to identify risk factors, and the adaptive approach (Section 3.5) will allow for the correction of animal welfare issues, while maintaining the project's objectives in balance.

### 3.5 Adaptive management and emergency plan

The IUCN Translocation Guidelines recommend developing an exit strategy as part of a translocation program (IUCN, 2013). Exit strategies are a well-established practice in business management but are rarely used in conservation (Ruiz-Miranda et al., 2020).

Exit strategies are not just responses to catastrophic failure but also to minor or major challenges and even success (e.g., what to do once project objectives have been achieved). Additionally, they are a way to prepare for new challenges and ensure that they can be addressed rationally. A well-planned exit strategy is a preventive tool against resource waste, the continuation of ineffective actions, and falling into cognitive biases, and it can help avoid detrimental consequences, legally, practically, and in terms of communication (Ruiz-Miranda et al., 2020).

In broad terms, an exit strategy can be triggered by: (1) success - project objectives have been conclusively achieved; (2) completion - the project's timeline or funding has come to an end; (3) failure - project objectives have not been achieved and are considered unattainable; or (4) voluntary cessation - one or more partners or stakeholders decide to end their commitment.

The translocation program of the LIFE Connect Ricotí project has a pre-established project timeline (2021-2026). Given the species' status and trend, and the experimental nature of the translocation program, it is unlikely that the ultimate goal of species recovery will be achieved before this deadline. Therefore, in general, the translocation program will be considered **successful** if, upon completion, the observed results of translocation and the projected estimates of population viability are such that the partners agree that a large-scale translocation program is likely to enhance the species' recovery prospects. It will be considered a **failure** if, at the end of the project or earlier, the observed results of translocations, practical and logistical issues encountered, and projected estimates of population

viability are such that the partners agree that the continuation of translocations is unlikely to improve or even jeopardize the species' recovery.

The translocation program developed as part of the LIFE Connect Ricotí project is (1) intended to learn about the dynamics of translocations and how to manage them and (2) informed by the results of the population viability analysis (PVA) which indicates both a limited impact of individual extraction and a poor baseline prognosis for the species even in the absence of translocations. This is especially important because, until now, translocations of the Dupont's lark have not been attempted, and while preliminary studies suggest that habitat restoration practices are effective, it cannot be known with absolute certainty if the decline factors will have been resolved when translocations occur (IUCN, 2013). Filling this knowledge gap is a fundamental goal of the LIFE Connect Ricotí project. In this sense, while failure is undesirable, this project explicitly aims to highlight the reasons for failure and possibly correct them. Therefore, technical challenges and low survival per se are not necessarily reasons to abandon the program. Instead, the partners will rationally assess the challenges and guide further actions. This may include additional experimental approaches, which could involve a direct trade-off between individual mortality and greater rational benefits at the program and species scale; in other words, actions suspected of failure may be carried out with the declared objective of accumulating more knowledge using an adaptive management approach (Canessa et al., 2016).

Possible specific reasons for failure include:

- Failure to capture, transport, and release the desired number of individuals which could be due to:
  - insufficient capture of birds at the source location, particularly females,
  - excessive mortality or complications during transportation,
  - failure to obtain approval for release after health checks (Section 4.1.5),
  - sudden sharp decline in and/or extinction of the source population,
  - lack of negotiation regarding the availability of release sites with local landowners.
- Failure to establish at the destination site, resulting from:
  - immediate dispersal of the birds far from the release site (<2 weeks after release), with or without successful return to the source population, and failure to establish even in nearby locations,
  - excessive short-term mortality at the release site (<1 year after release) due to predation, lack of food, or other traumatic events.
- Lack of persistence at the destination site in the medium term (before the end of the first breeding period after release), resulting from:
  - excessive mortality or dispersal of released individuals,
  - insufficient recruitment of new breeding individuals, due to mating failures, nesting, hatching, or fledging failures, or excessive mortality before new recruits can reproduce.

Possible translocation failures are predominantly related to the demographic dynamics of individuals and populations. Previous experience with capturing and handling the species suggests that unacceptable welfare issues are unlikely to arise during translocation (Section 3.4); in any case, such

issues would likely involve unsustainable levels of mortality, leading to a broader overall failure and triggering the need for review or withdrawal. The potential for socioeconomic repercussions is limited because the translocation component of this project does not involve permanent structures or specific employment for bird translocation (e.g., it does not entail a large ongoing captive breeding program with dedicated staff).

Immediate, evident complications can be directly addressed on-site or during the translocation sessions (e.g., obvious defects in the design of transport boxes that may directly relate to individual deaths or decisions not to release). Conversely, general changes in release protocols to address suspected but not evident issues should only be implemented after thorough review, considering that changes may reduce the statistical power of data analyses and compromise the ability to infer best practices in the medium term. Annual reviews of the protocol will include: (1) the analysis of all data and estimation of mortality/failure rates at each step of the translocation process; (2) re-running PVA simulations (Section 3.1) using updated parameters, including uncertainty, to determine the viability of both source and destination populations based on the latest knowledge; and (3) review of results by LIFE partners and the Scientific Committee and consideration of whether to continue and how to proceed.

For this final step, the available options include, among others: (a) continuing with the current translocation protocols, (b) implementing revised protocols, (c) applying both the current and revised protocols in an experimental comparative setup, (d) suspending releases for one or more years, and (e) discontinuing the translocation program. Within each of these options, particularly (d) and (e), some or all released birds may not be recaptured and returned to the source population.

At the current stage of the translocation program (January 2023), the following measures are planned for before the next annual review following the first translocation season, to be conducted in the winter of 2023, and subsequent monitoring during the spring:

- Confirm the evaluation cycle described above, define explicit responsibilities for data analysis, and set deadlines for analysis, review, and discussion, taking into account the LIFE programme's information obligations.
- Prepare the PVA computer program for rapid updating with the latest estimates in accordance with established timelines.
- When evaluating project results and considering reviews, apply good elicitation practices to avoid groupthink and biases.
- Agree on how challenges and failures can and should be communicated to partners, through social media and official channels, with specific reference to the project's learning objectives.
- Explicitly consider the possible dynamics by which different partners, stakeholders, or funders may react to challenges and failures.
- Develop a consensus communication strategy to explain the expected long-term reliance on species conservation.

# 4 TRANSLOCATION PROTOCOL

## 4.1 Capture

### 4.1.1 Season

### 4.1.2 Team

### 4.1.3 Capture methods

### 4.1.4 Data collection and sex and age determination

### 4.1.5 Clinical examinations and radio transmitter attachment

### 4.1.6 Selection of individuals for translocation and control

## 4.2 Transport

### 4.2.1 Logistics and timings

### 4.2.2 Transport boxes

## 4.3 Release

### 4.3.1 Data collection and preliminary examination

### 4.3.2 Release methods



## 4. TRANSLOCATION PROTOCOL

The translocation of Dupont's larks will occur in three phases: (1) Capture, data collection, and clinical examination (Section 4.1), (2) Transport (Section 4.2), and (3) Pre-release examination and Release (Section 4.3). The capture phase encompasses the period from the capture of an individual until its placement into the cardboard box for transport to the release site. The transport phase is defined as the period between the previous phase and the extraction of the bird from the box at the release site. Finally, the release phase occurs between the previous phase and the actual release of the individual.

For each individual, all three phases will be carried out on the same day, ideally within 6 hours and during the early hours of the day (before noon). In addition to the translocated individuals, a similar number of 'control' individuals will be captured and fitted with radio transmitters using the same methodology as in the donor populations (Section 4.1.6). These control individuals will be released at the same location in which they were captured. Both control and translocated birds will be held for the same duration (Section 4.1.6). The information obtained during the captures, along with their post-release monitoring, will allow us to assess the birds' condition, analyse behavioural differences between donor and recipient areas, and address any unforeseen issues during the translocations.

### 4.1 Capture

#### 4.1.1 Season

Captures for the translocations will take place between mid-December and early March, just before the peak reproductive period of the species (Section 1.1). Conducting translocations during this time may promote the settlement of translocated individuals, as demonstrated in similar studies (Brooke et al., 2020).

#### 4.1.2 Team

Captures will be carried out by personnel specialized in this type of activity and with extensive experience with the species. These personnel must be accredited with the corresponding permits for the capture and marking of wild fauna (Law 42/2007 on natural heritage and biodiversity) as well as training in animal experimentation (functions b, c, d; Order ECC/566/2015). Each capture team will require at least 3 individuals with the following roles: (1) setting and checking traps and extracting captured birds; (2) obtaining data from captured individuals, collecting biological samples, and fitting radio transmitters; (3) recording the information and verifying the **checklist** (Appendix 1.1). One team member will be responsible for regularly checking the traps (at least every 30 minutes) for extraction, repositioning, or removal, transporting captured individuals in clean cloth bags to the processing area located near the vehicle. Another team member will handle banding, data collection, and the collection of biological samples, as well as fitting radio transmitters to each bird. Finally, a third team member

will accurately record all information on data sheets and review the **checklist**. The health assessment can be conducted by the specialized technical personnel (person responsible for banding and data collection), although the presence of a veterinarian during the initial translocations is recommended to ensure the appropriateness of the procedure.

#### 4.1.3 Capture methods

Birds will be captured in the donor areas using mist nets (30 x 22 cm) (Pérez-Granados et al., 2022), which may be accompanied by a sound lure. These harmless traps are placed in territories or areas of interest previously identified and continuously monitored by the responsible personnel. This method is the most commonly used for capturing Dupont's larks, although the sex ratio in captures is heavily skewed towards males. If necessary due to logistical reasons, the nets can be set up the evening before, left inactive to prevent capturing any animals, and activated early in the morning on the following day.

Given the difficulty of capturing females of this species using this method (Vögeli et al., 2007), alternative techniques may be employed if the desired number of females is not captured during the translocation period. In such cases, effort will be made to locate and detect nests during the day or night using thermal cameras (e.g., *Pulsar Accolade 2 LRF XP50 Pro Binocular*), a circular net with a diameter of 60 cm, a 2.5 m pole, and a 5000 lumens flashlight (Redfern & Clark, 2001; Hughes et al., 2021). Once a nest is located, it will be georeferenced, and an attempt will be made to capture adult individuals during the day by placing several traps (2-3) without lures in the vicinity of the nest. During the breeding season, the captured birds will be fitted with programmable CTx radio transmitters (Lottek Ltd.) for tracking in the donor population and possible translocation before the following year's breeding season (Section 5).

The captured birds will be removed from the trap and temporarily placed in cotton bags (standard size ~20 x 25 cm) commonly used in scientific banding, where they will be kept in the absence of light and in a state of rest. The capture team will transport the bags with the captured individuals to the processing station, located near the vehicle, for data collection, clinical examination, radio transmitter fitting, and, if applicable, transportation. The bags will be used only once per bird per day and adequately disinfected before reuse to eliminate the presence of parasites or genetic material. If the temperature is below 10-12°C, the birds will be processed and held (Section 4.2) inside the vehicle, which should be maintained at approximately 10-12°C.

#### 4.1.4 Data collection and sex and age determination

During the processing of captured birds, they will undergo a clinical examination for inclusion or exclusion from the translocation program (Section 3.3.5). Biological samples will be collected as well as data on the bird's physical condition, variables related to the individual's personality, and potential effects resulting from capture, handling, and transportation. Each captured individual will have a



data sheet ('Capture Sheet'; Appendix 2.1), in which the collected information will be recorded. The sequence of procedures to be performed in the 'capture' phase is detailed in Appendix 1.1 ('Translocation Protocol Checklist'). The captured individuals will be handled for the shortest time possible.

In the field, the sex of the specimens will be determined through a discriminant function, i.e., males have a wing length >97 mm (Vögeli et al., 2007; Suárez, 2010). Subsequently, the sex of individuals will be genetically determined from the collected blood samples (Section 4.1.5). The determination of age of the specimens will be based on the state and colouration of the plumage. In the Dupont's lark, both juveniles and adults undergo a complete moult of their plumage after the breeding period, which makes them indistinguishable from that moment on. Therefore, during the breeding period, adults of unknown age (Euring code = 4) and specimens born during the current year's breeding period (Euring code = 1 if in a nest or 3 if a fledgling) can be identified, but once the moult has occurred (approximately in August), all specimens are of unknown age (Euring code = 2 until the end of the calendar year and 4 from then on), whether they are yearlings or older individuals. Given the uncertainty about the phenomenon of juvenile dispersal in this species, individuals will be captured and translocated just before the start of the breeding season to avoid inadvertently capturing dispersing juveniles.

#### 4.1.5 Clinical examinations and radio transmitter attachment

Each captured animal will undergo a comprehensive clinical examination, along with the collection of biological samples for the detection of relevant pathogens (as identified by the DRA; Section 3.3). Clinical examinations and sampling will be carried out by a specialist veterinarian at the time of capture.

During the subsequent processing after capture, standard biometric data will also be collected, such as wing length, third primary feather (providing information on the individual's migratory behaviour), tail, tarsus, and bill measurements.

The clinical examination will include:

- Weight (g).
- Subcutaneous fat code: code for fat accumulation according to Pinilla (2000).
- Muscle mass code: code for classification of pectoral muscle according to Pinilla (2000).
- Attitude/General appearance.
- Response to handling: number of times the bird struggles and attempts to escape ('struggles') during the first minute of processing. That is, from the moment it is taken out of the cotton bag-collector (used to transport the bird from the capture location to the processing location) until 1 minute of processing has elapsed.
- Presence of ectoparasites (mites, lice, ticks).
- Examination of eyes, ears, nostrils, bill, oral mucosa, cloacal mucosa, peri-cloacal area, skin, plumage, musculoskeletal system, and abdominal auscultation and palpation.

Individuals showing clinical signs of illness or not receiving a positive evaluation from the veterinarian will not proceed to the next phase of the translocation (transportation to the release area). In each case, based on the clinical examination and prior to potential translocation, it will be decided whether the individual will be: 1) transported for translocation; 2) released with a radio transmitter at the capture site (control individual); 3) released at the capture site without a radio transmitter; 4) transferred to an authorized recovery centre for rehabilitation; or 5) euthanized due to poor health.

Once this examination is completed, the veterinarian or qualified technician will issue a translocation decision that will determine whether sampling and the next phase of the translocation will proceed. Biological sample collection will consist of:

- Fresh faeces: faecal samples will be collected at the time the animal defecates. Probably the best time is during capture as they usually defecate when caught in the trap, making it easy to collect the faecal sac on the ground. Another option is to collect faeces after transportation to the release area, when the animal is taken out of the transport box and checked. The samples will be used for diet analysis.
- Ectoparasites: if necessary, samples of mites, lice, and/or ticks may be collected. Mites and ticks will be stored in a properly labelled *Eppendorf* tube with 99% ethanol. Lice can be collected by extracting 2-3 small body feathers and stored in an empty *Eppendorf* tube.
- Blood: a sample corresponding to 0.05% of the animal's body weight (50-100µL) will be taken via jugular puncture using a 28G needle and a 1 ml syringe and then stored in 99% ethanol. The samples will be used for sex determination of each animal, genetic studies, and the detection of DNA from hemoparasites (*Plasmodium*, *Haemoproteus*, *Lecocytozoon*) using PCR.
- At the end of the blood extraction, if necessary, the animal may be treated with a single bolus of 0.3 ml of physiological saline administered subcutaneously. This will help the animal replenish fluids lost during blood extraction and provide hydration during the transportation period prior to release.

The methodology used for collecting biometric data, biological samples, and the clinical examination is detailed in Appendix 2.1. Finally, the individuals selected for translocation or control will be equipped with a Coded radio transmitter (Section 5.2.1) before being introduced into the transport box (Section 4.2.2).

#### 4.1.6 Selection of individuals for translocation and control

Once selection and data collection have been completed, the individuals deemed suitable for translocation will be randomly classified for transport to the destination site ('translocated' individuals) or for release back into the area of origin ('control' individuals). The classification will be done separately by sex to ensure the release of an appropriate M:F ratio in both groups (Section 3.1), with priority given to the destination site in the case of uneven or insufficient numbers. All individuals will then be placed in cardboard transport boxes.

To minimize biases in analysis and comparison, it is essential that the protocols for VHF transmitter marking, release, and tracking, explained in the following sections, be applied identically to both groups. Ideally, the following protocol is recommended: the two capture groups are separated, with one group ('translocation' group) proceeding to the destination/release site, and the other group ('control' group) remaining in the vehicle and continuing to move towards the origin site. The two groups coordinate by phone until the 'translocation' group reports that they have reached the destination site. Then, both groups proceed to mark and release the individuals at their respective sites (Section 4.3).

## 4.2 Transport

### 4.2.1 Logistics and timings

Since the Dupont's lark is a territorial and non-gregarious species, individualized transportation of the captured birds is most suitable to avoid conspecific aggression (Lovegrove & Veitch, 1994; Parker, 2002; Brooke et al., 2020). As the translocations will be conducted within a short period (<6 hours), the design of the transport enclosure should minimize natural movement to reduce stress or the risk of injury (Sherwin, 2004; Gebhardt-Henrich & Steiger, 2006). In this regard, the individuals to be translocated can be transported in small compartments or separate boxes where they will be placed after processing and data collection (capture phase) (Withers et al., 2019). Following the system used in other projects, these boxes should be individual, well-ventilated, and opaque to external light (Bennett, 2012; Brooke et al., 2020).

Given the short transport time and recommendations from other similar projects (Raso lark translocation program; P. Geraldès, personal communication), it will not be necessary to feed the birds during transport. The total estimated travel time from capture to the destination is a maximum of 3 hours, with an additional maximum period of 2-2.5 hours before departure, as all birds are processed at the origin and fitted with transmitters, along with a maximum period of 0.5 hours at the time of release. In the case of delays exceeding 6 hours, the boxes should be opened to provide food in the form of Tenebrionidae larvae, used for baiting the capture traps. Upon reaching the destination site, the transport boxes will be unloaded from the vehicle and taken to the release area.

During or after transportation, the data contained in the 'Release Sheet' should be properly recorded (e.g., faeces, feathers; Appendix 2.1). To minimize the effects of temperature differences between capture site, transportation (vehicle), and release site, temperature will be measured at all three locations using a digital thermometer. This will prevent sudden temperature changes during transport and the retention and transportation of animals at low temperatures. To achieve this, a comfortable temperature of around 15°C will be maintained inside the vehicle.

### 4.2.2 Transport boxes

All birds selected for transportation, either to the translocation site or as control individuals to be released back at the origin site, will be transported by car to the destination using designated transport boxes (cardboard box, size: 12.5 x 10 x 8 cm; Figure 4.1). The individuals will be free inside the box. To prevent the birds' feet from slipping on the cardboard floor and enhance animal welfare, the floor of the box will be lined with a foam rubber sheet. The cardboard boxes with the birds will, in turn, be placed inside a larger plastic box (six cardboard boxes per plastic box), preventing free movement between them. For this purpose, the floor of the plastic box will be covered with a high-density polystyrene plate with different cutouts with the exact measurements of the cardboard boxes (Figure 4.1). The thickness of the polystyrene plate should not obstruct the ventilation holes in the boxes (Figure 4.1). Lastly, the plastic box containing the cardboard boxes with the birds will be covered with mesh shade cloth, reducing light entry while maintaining ventilation. The box should always be carried by two people, each having a free hand to prevent falls that could harm or crush the birds. The plastic box will be placed in the passenger area of the vehicle or in a visible compartment of the trunk, properly secured to allow monitoring during the journey. Special attention should be paid to weather conditions, as the birds may overheat inside the boxes.



**Figure 4.1.** Cardboard transport boxes contained within a plastic box, immobilized by a 2cm thick high-density polystyrene base. The cardboard boxes have five 15mm diameter holes on one of their sides, and the floor is covered with a foam rubber sheet to prevent the birds from slipping on the cardboard. The entire assembly is covered with a mesh shade cloth, reducing light entry into the boxes while maintaining ventilation.

## 4.3 Release

### 4.3.1 Data collection and preliminary examination

At the release site, each bird will be extracted one by one, taking necessary precautions to avoid any unintended escapes. Each animal will be re-examined for signs of disease or stress (Section 4.1.5). Once this examination is completed, a translocation decision will be made, determining whether the animal will be: (1) released at the destination site with a radio transmitter, (2) released at the destination site without a radio transmitter, (3) transferred to a recovery centre, or (4) euthanized due to poor health. In the case of a positive translocation decision, the bird will be released while the data on the 'Release Sheet' is being filled out according to the designed checklist (see Appendix 2.1). The radio transmitter, which will have been activated at the agreed-upon time for proper programming (Section 5.2.1), will be checked to ensure its proper functioning before releasing the bird. If euthanasia is necessary, the bird will be properly preserved for transport and subsequent necropsy at IREC-CSIC to determine the cause of its critical health condition.

### 4.3.2 Release methods

Finally, a technician will release the bird through hard release. They will place the bird on the ground and slowly back away, observing, along with the rest of the team, the bird's behaviour to record tonic immobility time, escape distance, and any other relevant aspect. All birds will be released successively at the same release point, not simultaneously.

Ideally, the birds will be released in the early morning or evening with good weather conditions. Prior to release, the weather forecast will be consulted. An early morning release provides the birds with ample daylight hours to find food and shelter before nightfall. During the release, any other person not directly involved in the release process should maintain a minimum distance of 20 metres. Throughout the entire release process, just like during capture and transportation, effort will be made to minimize noise to reduce stress on the birds.

# 5 MONITORING

5.1 Objectives

5.2 Tracking methods

5.2.1 Transmitters

5.2.2 Automatic radio telemetry station



## 5. MONITORING

### 5.1 Objectives

Post-release monitoring is crucial to measure the length of time individuals stay in the destination area and, therefore, the success of the translocations. Without this monitoring, it is not possible to evaluate the outcome of the translocations for future conservation projects (Parker et al., 2013). Additionally, proper monitoring allows for the estimation of survival rates of translocated individuals and the calculation or improvement of population viability parameters.

The monitoring should be related to the operational objectives of the translocation proposal. The design of post-release monitoring should align with the questions that need to be answered and the subsequent use intended for the data. Monitoring is necessary because there are many uncertainties about the translocation. For instance, monitoring may be more valuable if it is uncertain whether the habitat in the release site is too connected to adjacent unmanaged habitats, if there is high pressure from threats in the release site, or if habitat suitability is unclear. Post-release monitoring can be used to determine where translocations have failed, if a different management approach would prevent failure in the event the species is translocated to the same location again, and, if not, the viability of future translocations. For example, if monitoring shows only males, there may be an issue with dispersal or predation, or if there are breeding pairs, but all offspring have disappeared, there may be a problem with juvenile recruitment.

There are two aspects that present high uncertainty in translocations:

Anchoring. The first element of uncertainty relates to the settlement/movement of birds after release.

→ ***Will the translocated birds stay in the release area or attempt to return to the origin area or disperse?***

Although the Dupont's lark tends to stay in its breeding areas throughout the annual cycle, there are observations from outside the breeding period that suggest movements beyond the breeding season. This could mean that, naturally, translocated individuals make movements outside the release area, and it may not be solely due to the translocation itself. To control for this factor, individuals from the origin area that are not translocated will be marked with VHF Coded transmitters, and their movements will be monitored to determine if they make movements outside the marked area.

→ ***Are there methods to improve the success of anchoring?***

These methods may include simple measures (e.g., feeding and breeding) and stronger measures (e.g., soft release with aviaries). Given the limited number of available birds, the translocation will initially be conducted using a passive adaptive management approach (Runge, 2011), in which the method considered most effective a priori (soft release) will be employed, the outcomes will be monitored, and management will be modified if necessary.



Survival and Persistence. The second key uncertainty relates to demographic trends after release.

→ *Do the released individuals survive and reproduce sufficiently for the population to grow?*

Information on survival will be collected through the monitoring of radio-marked individuals, both in the translocation areas and in the control sites (see the following sections). Attempts will be made to locate evidence of reproduction associated with these individuals through nocturnal searches and nest location. The results will be compared with the survival and fecundity estimates used in the PVA projections (Sections 3.1 and 3.5), and those obtained in the control sites. These cross-comparisons will help determine the suitability of the habitat in the release areas and the translocation techniques. When analysing and evaluating the data, it should be noted that in most conservation translocations, some additional mortality can be expected in the immediate post-release phase (Panfylova et al., 2016; Armstrong et al., 2017).

## 5.2 Tracking methods

As the Dupont's lark is a scarce and elusive species, the tracking method cannot rely on direct observations. Its detectability outside the breeding period is very low, and its singing activity mainly occurs in the hour before sunrise. This low detectability, coupled with few recaptures of banded individuals and their light weight, restricts the methodologies to be used, making remote tracking through radio telemetry the most suitable option.

This method involves tagging the birds with VHF (very high frequency) Coded radio transmitters and installing automatic fixed receiving stations for signal detection. In parallel, manual (mobile) receivers will be used to detect individuals (translocated or control) that move away from the reception area of the fixed stations. The steps to follow in this post-release tracking protocol are shown in Appendix 1.2.

### 5.2.1 Transmitters

The captured specimens will be individually marked with a metal ring and VHF radio transmitters, as the small weight of this species currently does not allow for the use of GPS-GSM transmitters available on the market. The average weight of the Dupont's lark is 35g, so the radio transmitters used cannot exceed 3% of the bird's weight (Kenward, 2000). The harness used for fitting the VHF will be a pectoral harness with a thickness of 1 mm, made of nylon/Teflon (see Williamson and Witt, 2021 for more details). The measurements of the harness will be customized for each individual bird according to their body size.

Two types of VHF transmitters will be used depending on the time of year and/or the sex of the individual. On one hand, VHF Coded transmitters (NTQB2-4-2 Coded **Nanotag** of 0.9 g; Lotek Wireless Inc., Canada) will be used for the translocations, which will take place between mid-December and

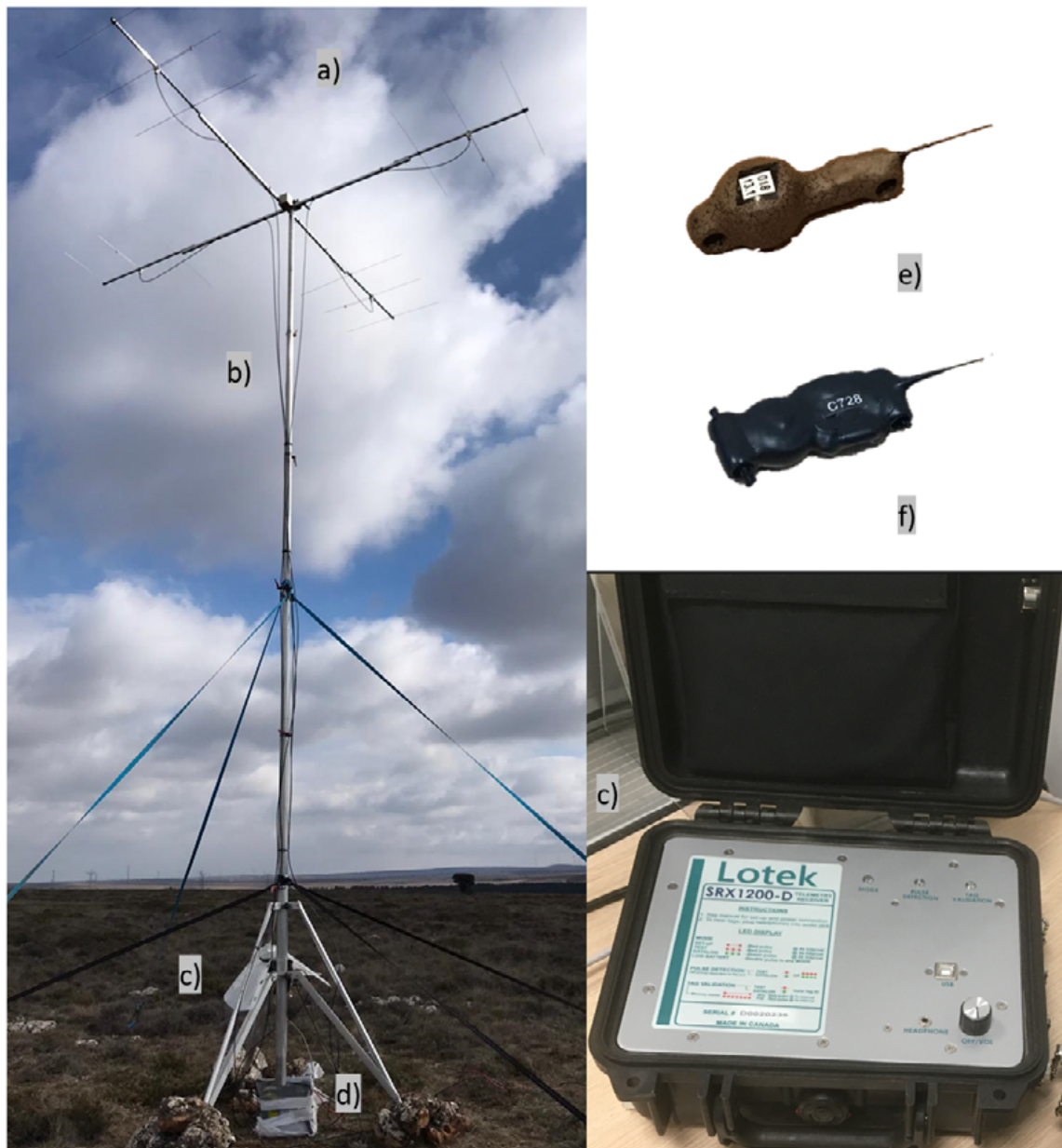
early March. These devices, unlike classic VHF or Beeper transmitters, emit at the same frequency but with different pulses, and have a much longer battery life than traditional Beeper transmitters, making them particularly suitable for the objectives of this project. To further extend the battery life of the Coded transmitters and align with the daily schedule of Dupont's larks, they will be programmed to transmit a signal every 13 seconds and will emit signals for 12 hours a day, during the peak flight activity of the species (approximately one hour before sunrise). This schedule requires adjusting the activation time of the Coded devices according to the time of year because of the time difference of sunrise between the translocation period and the breeding period. To have the Coded transmitters active during the breeding period during the peak activity hours of the species (3:30 to 15:30), they should be activated during the translocations for the period from 2:30 to 14:30 h. In this way, the estimated lifespan of the Coded transmitters will be 721 days, providing an extended period of tracking for the translocations and control individuals.

Given the difficulty of capturing this species, especially females, individuals can be marked, if necessary, during the breeding period (Section 4.1.3), to be detected (and translocated) during the following year's translocation period, before the start of the new breeding period. These individuals, primarily captured through nest location (Section 4.1.3) will be equipped with CTx **Connectivity** VHF Beeper Tag transmitters (Lotek Wireless Inc., Canada), using the same type of pectoral harness.

This device consists of a conventional VHF Beeper transmitter with the option to be programmed to emit on specific days (see Appendix 2.5). In this way, the battery life of the device can reach at least 1 year. This will allow individuals to be marked during the breeding period, mainly uncaptured females, and to be recaptured and translocated outside the breeding period (in the following calendar year). Once recaptured, the transmitters will be changed, and the Coded transmitter will be installed following the previously indicated procedure. For tracking birds marked with CTx radio transmitters, a portable multifrequency receiver connected to a foldable 3-element **Yaggi** antenna will be used, although, if necessary, automatic radio telemetry stations (Section 5.2.2) could be adapted for these devices.

### 5.2.2 Automatic radio telemetry station

In each translocation area and in the source zones, an automatic fixed radio telemetry station will be installed (Figure 5.1; Appendix 2.2). This station should be located at a central and elevated point in the area to facilitate the reception of the Coded signals. The selection of these points will consider the singing behaviour of the Dupont's lark, which can be performed in flight up to a height of 100-150 m, making signal detection easier. However, it is necessary for the antenna to also detect the transmitters when they are on the ground or among shrubs, a situation that is especially common in breeding females. For this reason, the station should not be installed at a point excessively elevated above the average height of the area. Preliminary tests have shown a high detection capacity of the antennas at distances greater than 2 km when the Coded transmitter is at a height (Navalpotro et al., in preparation). If the release area is very large, the installation of multiple stations is recommended.



**Figure 5.1.** Components of a tracking station: a) four 3-element *Yaggi* antennas, b) coaxial cables, c) SRX-1200D receiver, d) battery, and e) 0.9-gram *Nanotag* NTQB2-4-2 transmitter.

Each station is composed of a receiver, four 3-element *Yaggi* antennas (programmed with a central frequency of 150.100 MHz), four coaxial cables, and a power source (Figure 5.1). The optimal receiver for this type of tracking is the SRX1200-D from Lotek (Lotek Wireless Inc., Canada), which directly decodes the IDs of the Coded transmitters, generating a database of detections. The antennas are attached to a minimum 6-metre-high mast and placed at the same level, 90 degrees apart. Each receiver is connected to a 12V battery to power the system and a solar panel to provide autonomy to the battery for 2 weeks. Each receiver is connected to a modem with a mobile data card to enable remote connectivity.

## 5.3 Tracking

### 5.3.1 Tracking within the study area

Post-release tracking will be carried out automatically using the automatic radio telemetry stations. These stations consist of a fixed reception system capable of automatically receiving data from the transmitters fitted on each individual. These receivers will store a basic data file (transmitter number, date and time of emission, reception antenna, and signal strength) that will be remotely sent through a Wi-Fi router to a computer.

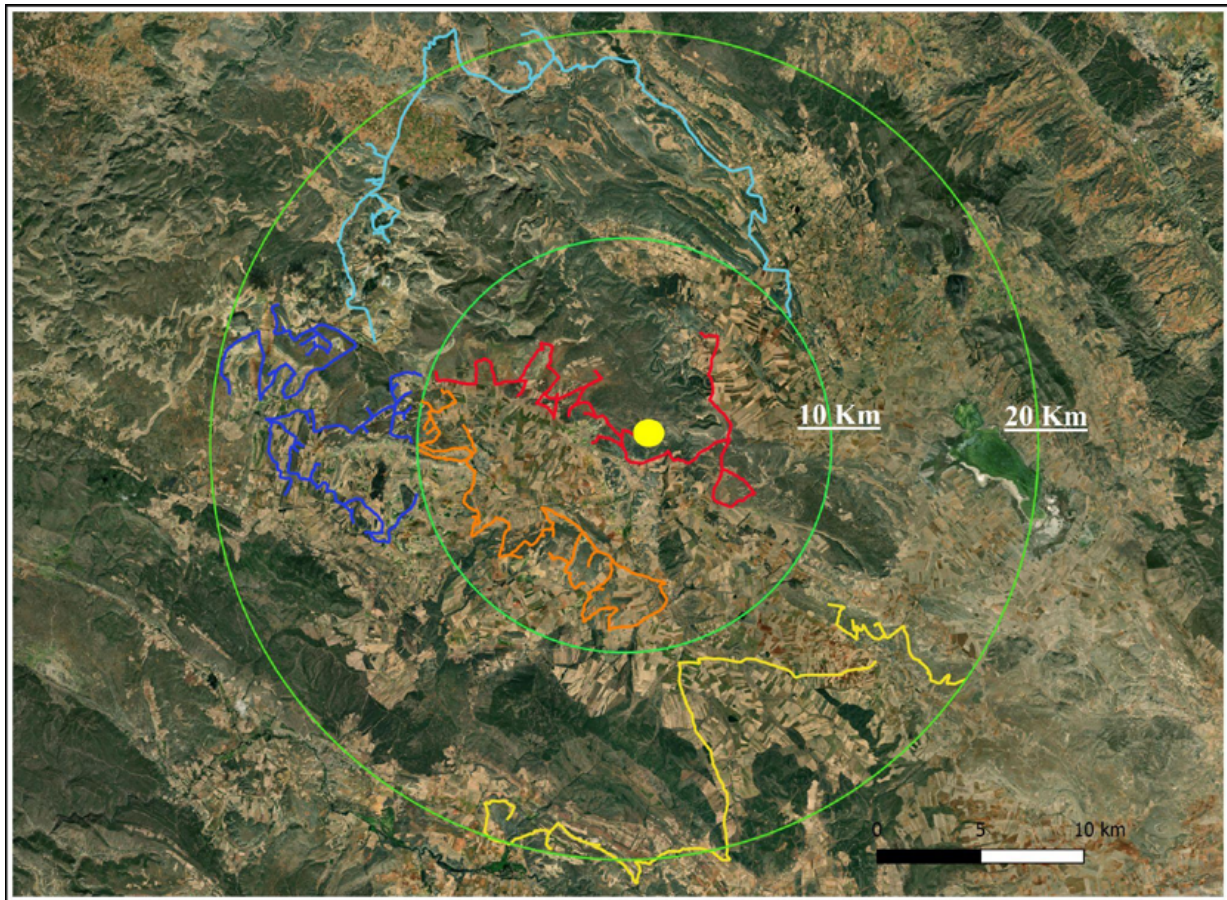
Periodic visits to the stations will be made to assess their correct installation and functioning and to perform battery replacements, if necessary. All modifications of settings, repairs, battery changes, and operational visits will be recorded in the 'Station Revision and Data Download Sheet' (Appendix 2.3). During each visit, individuals will be detected using the handheld manual receiver (Lotek SRX-1200M; Lotek Wireless Inc, Canada) to verify the correct recording of the station data. The manual receivers have the option to store 120 seconds of continuous data when the **Quick Record** function is activated. This command creates a file with a record of the detected devices during the search. In the event that nothing is detected, the created file will be empty. The manual receivers also have the option to record the GPS point from where the search is being conducted.

The database of all receivers will be reviewed every three days to check whether the individuals remain within the release area. In addition to the files stored in the receiver, the individuals that have been detected and the search time for each visit will be recorded in the 'Manual Tracking for Marked Individuals Sheet' (see Appendix 2.4).

### 5.3.2 Tracking outside the study area

In the event that no radiomarked individuals are detected through the automatic recording of the stations or during visits, intensive searches will be conducted in the vicinity of the translocation area or the source population (in the case of control individuals or potentially returned individuals). This search will be carried out from a vehicle and will involve stopping periodically in areas of potentially suitable habitat, mapped in two sectors: one within a radius of 10 km and the other 20 km from the tracking station (see example in Figure 5.2). The search will start at the nearest point to the fixed tracking station and will gradually expand outwards, progressively moving away from that point, until covering the first 10 km area. The search will take place from one hour before sunrise (when the birds are most active) until the transmitter stops emitting a signal, 12 hours later.

For this search, a handheld manual receiver connected to a directional antenna, placed on a four-metre pole, will be used, anchored to the car's hood. At each stop, a 360° turn with the pole will be performed so that the receiver covers the entire area.



**Figure 5.2.** Rescue map prepared for the Embid site. The two green circles represent the search areas of 10 and 20 km. The yellow point corresponds to the location of the fixed tracking station. The coloured lines represent the tracking transects.

The handheld manual receiver used for detecting individuals outside the detection range of fixed receivers will be a Lotek SRX-1200M receiver (Lotek Wireless Inc, Canada) with a portable antenna. This receiver operates with rechargeable 'C' type batteries and has a mode that allows continuous collection and autonomous data recording during the scanning operation. For optimal data collection, echo and pulse amplitude filters can be applied, and the gain can be adjusted to receive the maximum signal without collecting noise. Since all Coded transmitters have the same frequency, the receiver loops through the same channel. During the search and scanning with the handheld receiver, the GPS location must be recorded at all times, generating a route or track that can be related to the collected data. A 'Manual Tracking of Marked Individuals Sheet' (see Appendix 2.4) has been created to record the zones and time dedicated to each transect of the marked individuals.

If manual ground searches at different sites are not sufficient, the portable receiver will be left in an elevated area with optimal habitat for a few days to try to detect any individual that was not located during the initial search. These tracking stations will be portable, and the antennas and the 6-metre mast will be attached to a folding tripod, with four antennas secured at the maximum height. Another technique that can be used in the case of transmitter loss is the use of a drone to elevate a SensorGnome-type radio receiver and undertake detection from a higher altitude (30-50 m). In tests conducted to find the best tracking method (Navalpotro et al., in preparation), it was observed that as the reception height increases, the detection distance also increases. This tracking will be conducted for 10 minutes at each point and at different points progressively farther from the marking area.



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## LIFE CONNECT RICOTÍ: TRANSLOCATION PROTOCOL CHECKLIST

**Preparation the evening before the captures**

- If necessary, set up traps (kit of 3 traps + playback) the previous evening at the pre-identified sites. Install 3-4 kits per team (2 teams)
- Ensure that the car's fuel tank is full

**Preparation before heading to the capture site (check the night before)**

- Check capture equipment (x2 teams):
  - Capture/transport/release sheets
  - Traps (in case more units need to be replenished or placed)
  - Playback
  - Collector bags
  - Collector bag tags
  - Ringing box (balance, callipers, pliers, rulers, rings, etc.)
  - Mealworms
  - Handheld GPS
- Check sampling equipment (x2 teams):
  - Syringes
  - Capillary tubes
  - Cotton
  - Eppendorf tubes with 99% alcohol and a rack
  - Empty Eppendorf tubes
  - Eppendorf tube labels
  - Clean glass slides and racks
  - Paper envelopes
  - Mechanical pencils, pencils, and pens
  - Permanent markers
  - Tweezers for *mallophaga* and ticks
  - Cotton swabs (for mites)
- Check transportation and release equipment:
  - Thermometer
  - Individual cardboard transport boxes
  - Label stickers for cardboard boxes
  - Plastic box for arranging individual boxes
  - Shade netting
  - Straps and padding for boxes
  - Radio transmitters
  - Activation of Coded transmitters
  - Receiver + batteries and portable antenna
  - Prepared harnesses
  - Nail polish
  - Tweezers and scissors
  - Crochet needle
  - Loctite adhesive
- Check marking equipment:
  - Assembly and preparation of the harnesses
- Check documentation and licenses and place them in the cars





**Capture - processing**

- Appoint a technician to be in charge of reviewing this checklist
- While the capture teams are out, the base team prepares the processing site
- Check Coded radio transmitters and ensure they are emitting correctly
- Once the first capture is made, record the 'START TIME' on the capture form to track the capture time
- Remove the bird from the cloth bag and count the number of times the bird moves (attempts to escape) while in the hand of the ringer for 1 minute (i.e., the number of 'flutters')
- Ringing and biometric measurements
- Collect biological samples and store waste materials
- Attach the Coded radio transmitter to the bird
- Place the bird inside the car in the cardboard transport box and immobilize it in the plastic box (where all the cardboard boxes will be placed) and label the box. Cover the plastic box with shade netting
- Measure the ambient temperature. If it is below 10-12°C, the car's internal temperature should be adjusted to around 10-12°C to process the birds inside and during the retention period
- Record 'END TIME' on the capture form
- After 1.5 hours have passed, check the traps one last time and collect them (the capture + processing time should not exceed 2 hours)

- Process the last captured birds while collecting all the equipment
- Place the last processed birds in the cardboard boxes
- Inspect the condition of the birds before placing them in the boxes and make the 'translocation decision': exit strategy, control individual, individual for translocation
- Check that the capture forms are properly filled out
- Properly arrange (straps and padding) the cardboard boxes containing the birds inside the large plastic box and secure them for the car journey

**Transport**

- Complete the transport form with departure details (record 'START TIME')
- Provide food to the birds if the delay exceeds 6 hours from capture
- Measure the temperature inside the vehicle. If it is below 10-12°C, the car's temperature should be adjusted to around 10-12°C during transport of the birds
- During transportation, ensure that the boxes are secured, and everything is in order (do not open the boxes containing the birds)

**Release**

- Arrival at the release site
- Record 'START TIME' on the release form and 'END TIME' on the transport form
- Note the ambient temperature on the release form
- Review the release form
- Remove the first individual from its box and count the number of times the bird moves (attempts to escape) while in the hand of the technician releasing the individual for 1 minute (i.e., the number of 'flutters')
- Inspect the condition of the extracted birds (one by one) previously stored in the boxes and make the 'translocation decision' for each one: release with radio transmitter, release without radio transmitter, euthanize
- Check that the transmitter emits correctly (it only emits between 2:40 am and 2:40 pm)
- Proceed with the release by placing the bird on the ground at the chosen point
- Prepare a stopwatch to measure tonic immobility
- Measure tonic immobility (up to 30 seconds maximum) and escape distance
- Count and collect feathers and faeces from the transport box
- Dispose of the transport box

**Post-release**

- Disinfect material as deemed necessary
- Store the used cloth bag in a sack for washing

# LIFE CONNECT RICOTÍ: POST-RELEASE MONITORING CHECKLIST

## Preparation before field installation

- Configuration of settings for all receivers
- Configuration of the modem and remote control

## Field antenna installation

- Check material for the receiving station:

- Wi-Fi router with SIM card and cable
- Battery or Akku with cable
- Receivers
- Cables (4x per station)
- 12V battery
- Solar panel
- Solar transformer
- Four antennas, one for each receiver
- Cross-shaped poles for antenna installation
- Metal connector with holes (90°)
- Black piece for connector-tripod
- Central mast
- Metal legs (3x per central pole)
- Triangular piece to connect legs and mast (2x)
- Drill
- Disk and disk adapter
- Silver adhesive tape to protect the disk
- Rope (approximately 8 m per station)
- Car tie strap
- Pecks (3x)
- Strong ties to attach the black box to the mast and shade unit
- White and red tape to mark legs
- Toolbox with screwdriver and multiple keys
- Scissors
- Screws for the black piece
- Screws for tripod legs
- Screws for antennas

- Hardware installation:

- Antennas and cables connected
- Receiver powered on
- Battery charged
- Solar panel connected
- Wi-Fi modem activated

- Check remote operation and tag detection

- Fill out the installation sheet

## Weekly visits to each station

- Check the status of the station:

- Fill out the review and data download sheet
- Check:
  - Cables
  - Battery
  - Receiver
  - Modem

- Download data (if reported as necessary)  
Fill out the review and data download sheet

- Charge the battery if the voltage is below 12V



**Searches in case of signal loss from a transmitter**

- Commence the search along the pre-designed transects within a 10 km radius of the release point
  - If the animal is not detected, initiate the search along the pre-designed transects between 10 km and 20 km from the release point
  - Conduct searches every 250-500 m along each transect, with stops at high visibility spots (for 5-10 min) using the directional antenna attached to a 4 m pole.
  - For both searches, employ the portable (manual) receiver and record the routes taken (track) and the locations where the individual's signal was received
  - If the animal moves from one area to another, record the track ID and file name
  - Change frequencies every 5 minutes if there are tags with different frequencies
- In the case of an unsuccessful search with the above methods, use a drone to elevate a SensorGnome radio receiver to detect signals from a higher altitude (30-50 m)
  - Fill out the manual tracking sheet with the required data
  - Once all areas are searched or the transmitter is found, notify other groups, if applicable, and conclude the search

## CAPTURE SHEET

(Data obtained between the moment of capture and entry into the transport box)

N° CODE	METAL RING	RECAPTURE YES <input type="checkbox"/> NO <input type="checkbox"/>	DATE / / 2023	CAPTURE TIME	END TIME	CAPTURE COORDINATES					
No. BOX	COLOUR RINGS / POSITIONS	CAPTURE TECHN.	TEMP.	PLACE / MUNICIPALITY			LOCATION OF ORIGIN				
MEASURER	SEX	DATE	WING	F8 LENGTH	TAIL	TARSUS	MUSC	FAT	BROOD PATCH	WEITH	N° FLUTTERS (1 min):
BILL-NOSTRIL	BILL-SKULL	BILL HEIGHT	BILL WIDTH	OBSERVATIONS:							
PHYSICAL EXAMINATION											
GENETIS YES <input type="checkbox"/> NO <input type="checkbox"/>	SMEARS YES <input type="checkbox"/> NO <input type="checkbox"/>	FAECES YES <input type="checkbox"/> NO <input type="checkbox"/>	FEATHERS YES <input type="checkbox"/> NO <input type="checkbox"/>	TICKS YES <input type="checkbox"/> NO <input type="checkbox"/> N°	WING MITES <input type="checkbox"/> S <input type="checkbox"/> O N°	EYE MITES YES <input type="checkbox"/> NO <input type="checkbox"/> N°	MALLOPHAGA YES <input type="checkbox"/> NO <input type="checkbox"/> N°				
TAG CODE	TAG FREQ.	TAG PULSE	TRANSLOCATION DECISION Animal to be transported for translocation ..... <input type="checkbox"/> Animal with radio transmitter released instead of captured (CONTROL individual) ..... <input type="checkbox"/> Animal released instead of captured without radio transmitter ..... <input type="checkbox"/> Transferred to a recovery centre ..... <input type="checkbox"/> Animal EUTHANASED due to poor health ..... <input type="checkbox"/>								
SKELETAL DEFORMITIES											

## TRANSPORT SHEET

(Data obtained during the time the bird is inside the transport box)

N° CODE	METAL RING	N° BOX	LOCATION OF ORIGIN	LOCATION OF DESTINATION	DATE  / / 2023	START TIME	END TIME	N° FEATHERS
FAECES YES <input type="checkbox"/> NO <input type="checkbox"/>		ENVIRON. TEMP.	CAR TEMP.	OBSERVATIONS:				

## RELEASE SHEET

(Data obtained from the time the bird is removed from the transport box until it is released)

N° CODE	METAL RING	START TIME	END TIME	DATE  / / 2023	LOCATION OF ORIGIN	LOCATION OF DESTINATION	
COORD. LIBERAC.	TAG EMITE SI <input type="checkbox"/> NO <input type="checkbox"/>		TECHNICIAN	No. FLUTTERS (1 min)	TONIC IMMOB. (30 sec max.)	ESC. DIST. (m)	TEMP.
OBSERVATIONS:			TRANSLOCATION DECISION				
			Animal released in destination location WITH radio transmitter (TRANSLOCATION) ..... <input type="checkbox"/>				
			Animal released in destination location without radio transmitter ..... <input type="checkbox"/>				
			Transfer to a recovery centre ..... <input type="checkbox"/>				
				Animal EUTHANASED due to poor health ..... <input type="checkbox"/>			

# INSTRUCTIONS FOR DATA COLLECTION USING THE CAPTURE/ TRANSPORT/RELEASE SHEETS

## CAPTURE SHEET

**No. CODE:** Unique code for each capture and date.

**METAL RING:** Alphanumeric code inscribed on the ring worn by the bird.

**RECAPTURE (YES/NO):** If the captured bird already has a ring when it is captured.

**DATE:** Date of the day the capture is made.

**CAPTURE TIME:** Time at which the capture is made.

**END TIME:** Time at which the captured bird is introduced into the transport box (after being processed).

**CAPTURE COORDINATES:** UTM coordinates of capture site (ETRS 89).

**No. BOX:** Code of the box in which the bird is transported. This code must also appear visibly on the outside of the box.

**COLOUR RINGS/POSITIONS:** If used, colours and positions in which the coloured and metal rings are placed on the legs. Use distance reading mark coding standards (Pinilla, 2000).

**CAPTURE TECHN.:** Technique used to capture the bird.

**TEMP.:** Temperature (°C) in the place where the bird is processed.

**PLACE / MUNICIPALITY:** Name of the area/municipal area where the bird is captured.

**LOCATION OF ORIGIN:** Location where the capture is made.

**MEASURER:** Person who performs the measurements, takes the biological samples and attaches the radio transmitter to the bird.

**SEX:** Sex of the bird according to the EURING code.

**AGE:** Age of the bird according to the EURING code.

**WING:** Maximum wing length ( $\pm 0.1$  mm) measured according to Svensson (1992).

**F8 LENGTH:** Length of the third primary of a wing ( $\pm 0.1$  mm) measured according to Pinilla (2000).

**TAIL:** Tail length ( $\pm 0.1$  mm) measured according to Pinilla (2000).

**TARSUS:** Tarsus length ( $\pm 0.1$  mm) measured according to Svensson (1992).

**MUSC:** Pectoral muscle classification code according to Pinilla (2000).

**FAT:** Fat accumulation code according to Pinilla (2000).

**BROOD PATCH:** State of development of the brood patch according to Pinilla (2000).



**WEIGHT:** Bird weight ( $\pm 0.1$  g) measured with a precision digital scale.

**No. FLUTTERS:** Number of times the bird scrambles and tries to escape during the first minute of processing. That is, from the time it leaves the cloth bag - collector (used to take the bird from the place of capture to the place of processing) until 1 minute after processing.

**BILL-NOSTRIL:** Bill length ( $\pm 0.1$  mm) from its end to the beginning of the nostrils.

**BILL-SKULL:** Total length of the culmen ( $\pm 0.1$  mm) from the beginning of the horny part of the bill (where it attaches to the skull) in a straight line to the tip of the upper maxilla.

**BILL HEIGHT:** Bill height ( $\pm 0.1$  mm) measured from the most distal part of the edge of the nostrils.

**BILL WIDTH:** Width of the bill ( $\pm 0.1$  mm) at the height of the nostrils.

**OBSERVATIONS:** Observations or data of interest not included in other sections of the sheet.

**GENETICS (YES/NO):** If a blood sample is obtained from the captured bird YES/NO. This will be extracted by jugular or brachial puncture. A maximum volume of 100 $\mu$ l will be extracted and stored in an Eppendorf container with 99% ethanol duly labelled with "Blood + Code number".

**SMEARS (YES/NO):** If smears are made YES/NO. If carried out, they must be fixed with 99% ethanol in a maximum period of 10 hours. See method of sample preparation: <https://www.youtube.com/watch?v=cI9GOtT73LY>. The smear must be labelled with the "No. Code".

**FAECES (YES/NO):** Collection of a faeces sample during capture YES/NO. The sample will be collected avoiding direct contact with hands to prevent contamination. It will be stored in an Eppendorf tube with 99% ethanol, properly labelled with "Capture faeces + Code number" to indicate that the sample was taken during the capture period.

**FEATHERS (YES/NO):** Collection of a body feather sample (not flight feather) YES/NO. The sample will be stored in an empty Eppendorf tube properly labelled with "Capture feathers + Code number" to indicate that the sample was taken during the capture period.

**TICKS:** Collection of tick sample YES/NO and estimation of the number of ticks on the head. They will be stored in an Eppendorf tube with 99% ethanol properly labelled with "Ticks + Code number."

**WING MITES:** Collection of a mite sample YES/NO and estimation of the number on the flight feathers of a single wing. They will be stored in an Eppendorf tube with 99% ethanol properly labelled with "Mites primaries + Code number."

**EYE MITES:** Collection of a mite sample on eye rims YES/NO and estimation of the number around both eyes. They will be stored in an Eppendorf tube with 99% ethanol properly labelled with "Mites eyes + Code number."

**MALLOPHAGA:** Sampling of mallophages YES/NO and estimation of the number on breast and rump feathers. It will be stored in an empty Eppendorf tube properly labelled with "Mallophages + Code number".



**TAG CODE:** Individual code of the radio transmitter attached in the individual.

**TAG FREQ:** Frequency of the radio transmitter attached in the individual.

**TAG PULSE:** Pulse of the radio transmitter fitted on the individual. As they are 'coded' radio transmitters, the frequencies can be the same between various devices, with the pulse of the emitted signal varying between them.

**SKELETAL DEFORMITIES:** Annotation of any malformations or lesions detected on the captured bird.

**TRANSLOCATION DECISION:** Based on the pre-established plan and the health status of the bird (once it has been processed), the decision about its fate will be made among three options: 1) animal to be transported for translocation; 2) animal with radio transmitter released instead of captured (CONTROL individual); 3) animal released at the capture site without a radio transmitter; 4) animal transferred to a recovery centre; 5) animal to be EUTHANIZED due to poor health.



FICHA TRANSPORTE

**No. CODE:** Unique code for each capture and date.

**METAL RING:** Alphanumeric code inscribed on the ring that the bird carries.

**No. BOX:** Code of the box in which the bird is transported. This code must also appear visibly on the outside of the box.

**LOCATION OF ORIGIN:** Location where the capture is made.

**LOCATION OF DESTINATION:** Location where the bird is intended to be released. In the case of a control, both locations must be the same.

**DATE:** Date on which the bird is transported.

**START TIME:** Time at which the bird is introduced into the transport box. It must be the same time as the 'End time' on the capture sheet.

**END TIME:** Time at which the bird is removed from the box in order to fit the radio transmitter.

**No. FEATHERS:** Number of feathers found in the transport box. Feathers will be counted after the bird has been removed from the box at the release site and has been released. They will be stored in a labelled paper envelope as "Transport feathers + Code number" to indicate that the sample was taken during the transportation period.

**FAECES (YES/NO):** Collection of faecal sample during transportation YES/NO. The sample will be collected from the transport box avoiding direct contact with hands to prevent contamination. It will be stored in a labelled Eppendorf tube with 99% ethanol properly labelled as "Transport faeces + Code number" to indicate that the sample was taken during the transportation period. If no faecal sample has been taken, it is understood that the bird did not defecate during transportation.

**ENVIRONMENTAL TEMPERATURE:** Air temperature before starting the transportation process.

**CAR TEMP.:** Vehicle temperature during transport.

**OBSERVATIONS:** Include any important data or issues during the transportation process.

RELEASE SHEET

**No. CODE:** Unique code for each capture and date.

**METAL RING:** Alphanumeric code inscribed on the ring that the bird carries.

**START TIME:** Time at which the bird is removed from the transport box at the release site. It must be the same time as the 'End time' on the transport sheet.

**END TIME:** Time at which the bird is released in the previously determined place.

**DATA:** Date on which the bird is released.

**LOCATION OF ORIGIN:** Locality from which the individual comes (locality of origin).

**LOCATION OF DESTINATION:** Location where the bird is released. In the case of a control, both locations must be the same.

**RELEASE COORD.:** UTM coordinates of the place where the bird is released (ETRS 89).

**TAG EMIT (YES/NO):** Verification of the proper functioning of the device before being fitted on the bird. To extend the battery life of the radio transmitters, they will only emit signals between 6:00 a.m. and 9:00 a.m. Therefore, the activation and verification of their proper functioning should be done within this time period.

**TECHNICIAN:** Person who has carried out the clinical examination and release of the bird.

**No. FLUTTER:** Number of times the bird scrambles and tries to escape during the first minute of the release period. That is, from the time it leaves the transport box until 1 minute has passed.

**TONIC IMMOB. (Min):** Tonic immobility calculated as the time elapsed from when the bird is released (placed on the ground) until it makes its first escape movement, either by walking or flying. If, after the first 30 seconds, the bird has not fled, it will be stimulated to elicit an escape response.

**ESC. DIST. (m):** Escape distance estimated by sight, as the distance that the bird flees in the first movement after its release.

**TEMPERAT:** Air temperature at the release site.

**OBSERVATIONS:** Observations or data of interest not included in other sections of the sheet.

**TRANSLOCATION DECISION:** Based on the pre-established plan and the health status of the bird after transportation, the decision will be made among three options: 1) animal to be released at the destination site WITH a radio transmitter (TRANSLOCATION); 2) animal to be released at the destination site WITHOUT a radio transmitter; 3) animal transferred to a recovery centre; 4) animal to be EUTHANIZED due to poor health.

REFERENCES

Pinilla, J. (2000). Manual para el anillamiento científico de aves. Madrid: SEO/BirdLife y DGCN-MIMAM.

Svenson, L. (1992). Identification Guide to European Passerines. Stockholm.



COMMON DATA INSTALLATION OF LOTEK STATIONS											Station ID
Date	Start time	End time	Technician	Zone	Coordinates			Installation			

Receiver ID	Modem		Scan settings		Channels			Antennas			Codelog started time	Comments
	SIM	IP	Scan time (13.6s)	GPS clock	N° of channels	Freq.	Type	Master option	Orientation correct?	Gain.		

COMMON DATA INSTALLATION OF LOTEK STATIONS											Station ID
Date	Start time	End time	Technician	Zone	Coordinates			Installation			

Receiver ID	Modem		Scan settings		Channels			Antennas			Codelog started time	Comments
	SIM	IP	Scan time (13.6s)	GPS clock	N° of channels	Freq.	Type	Master option	Orientation correct?	Gain.		

COMMON DATA INSTALLATION OF LOTEK STATIONS											Station ID
Date	Start time	End time	Technician	Zone	Coordinates			Installation			

Receiver ID	Modem		Scan settings		Channels			Antennas			Codelog started time	Comments
	SIM	IP	Scan time (13.6s)	GPS clock	N° of channels	Freq.	Type	Master option	Orientation correct?	Gain.		

COMMON DATA INSTALLATION OF LOTEK STATIONS								Station ID
Date	Start time	End time	Technician	Zone	Coordinates	Installation		

Receiver ID	Modem		Scan settings		Channels			Antennas			Codelog started time	Comments
	SIM	IP	Scan time (13.6s)	GPS clock	N° of channels	Freq.	Type	Master option	Orientation correct?	Gain.		

COMMON DATA INSTALLATION OF LOTEK STATIONS								Station ID
Date	Start time	End time	Technician	Zone	Coordinates	Installation		

Receiver ID	Modem		Scan settings		Channels			Antennas			Codelog started time	Comments
	SIM	IP	Scan time (13.6s)	GPS clock	N° of channels	Freq.	Type	Master option	Orientation correct?	Gain.		

COMMON DATA INSTALLATION OF LOTEK STATIONS								Station ID
Date	Start time	End time	Technician	Zone	Coordinates	Installation		

Receiver ID	Modem		Scan settings		Channels			Antennas			Codelog started time	Comments
	SIM	IP	Scan time (13.6s)	GPS clock	N° of channels	Freq.	Type	Master option	Orientation correct?	Gain.		

## INSTRUCTIONS FOR DATA COLLECTION

**DATE:** date of station and antenna installation.

**START TIME:** time when the installation process begins.

**END TIME:** time when the installation process ends.

**TECHNICIAN:** names of the working personnel.

**ZONE:** area where the antenna is installed.

**COORDINATES:** coordinates of the installation point.

**INSTALLATION:** fixed or mobile, depending on the type of structure.

**STATION ID:** note down the station code.

**RECEIVER ID:** receiver code.

**MODEM:** SIM ID and modem IP for remote connection.

### **SCAN SETTINGS:**

**Scan time:** it is recommended that 0.5 s be added to the tag interval (in our case if it is  $13.1 + 0.5 = 13.6$ ).

**Enable GPS clock:** mark this option for clock synchronization.

### **CHANNELS:**

**No. Channels:** number of channels used in this station.

**Frequencies:** scanned frequencies.

**Type:** tag type (Beeper or Coded).

### **ANTENNAS:**

**Master option:** note whether it's set as Master or not.

**Orientation correct?:** verify that Antenna 1 is facing north.

**Gain:** note the range.

**CODELOG START TIME:** time when the Codelog mode was activated.

**COMMENTS:** other important notes to be recorded.

## CHECKLIST OF MATERIALS NEEDED FOR THE RECEIVING STATION

Type	Item	Number
Receiver box	WIFI router with SIM card and cable	1
	Battery or Akku with cable	1
	Receivers	1
Cables	Cables for each station	4
Battery	Battery 12V	1
	Solar panel	1
	Solar transformer	1
Antenna	Antennas, one for each receiver	4
	Poles to set up the antennas as a cross	2
	90° metal piece with holes	1
	Black piece to attach 90° metal piece to mast or tripod	1
Tripod	Mast	1
	Metal legs for mast	3
	Triangle piece to attach the metal legs to the mast	2
	Drill if wanting to set the mast in the ground	1
	Disc and disc adaptor	1
	Silver tape to protect the disk	some
	Rope (approximately 8m needed for each station if car ropes are used)	8 m / station
	Car tie strap	3 /station
	Pecks	3
	Durable tie wraps to hold attach the black box to the pole and the shadow unit	>3 /station
	Red and white tape to mark legs and car tie straps	
	Tool box with screwdriver and tool to turn screws of the mast	1
	Scissors	1
Screws: 3 different types for each station	Screws for black piece to attach 90° metal piece to mast or tripod	2
	Screws to attach metal poles (cross) to 90° piece	2
	Screws to attach antennas to metal poles (cross)	8



Date	Technician	Station ID	Review				Data download			Start time	Comments
			Change battery	Repairs	Change settings	Other	Yes/No	File name	Start day – End Day		
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Date	Technician	Station ID	Review				Data download			Start time	Comments
			Change battery	Repairs	Change settings	Other	Yes/No	File name	Start day - End Day		
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INSTRUCTIONS FOR DATA COLLECTION

**DATE:** date the antenna is checked.

**TECHNICIAN:** names of the workers.

**STATION ID:** note station code.

**REVIEW:** note what kind of review has been done.

**Battery:** check the battery voltage and write it down.

**Repairs:** note if any repairs have been made.

**Change settings:** note if changes have been made to the settings.

**Other:** other revisions that may have been made.

**DATA DOWNLOAD:** note whether the data has been downloaded.

**Yes/No:** note if data has been downloaded when visiting the station.

**Remote:** note if the download has been remote.

**File name:** name of the file when saving it, also its extension.

**Start and end date of the data collected.**

**START TIME:** if it has been turned off to change the battery, write down the restart time.

id	Date	Technician	Receiver ID	Visit					Detections					Comments	
				Start time	End time	Zone or Track ID	X	Y	Freq.	H start	H end	Tag	De-grees		Neg.
														<input type="checkbox"/>	
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id	Date	Technician	Receiver ID	Visit					Detections						Comments
				Start time	End time	Zone or Track ID	X	Y	Freq.	H start	H end	Tag	Degrees	Neg.	
														<input type="checkbox"/>	
														<input type="checkbox"/>	
														<input type="checkbox"/>	
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														<input type="checkbox"/>	

INSTRUCTIONS FOR DATA COLLECTION

**ID:** detection ID.

**DATE:** date the search site is visited.

**TECHNICIAN:** names of the workers.

**RECEIVER ID:** receiver code.

**VISIT:**

**START TIME:** search start time to measure effort.

**END TIME:** end time of search process.

**ZonR or Track ID:** ID of the search zone or ID of the track that is recorded.

**COORDINATES:** coordinates of the point that is searched if it is not registered on the 'good areas' map.

**DETECTIONS:** data of the searches by frequency that is made and if there is detection or not.

**FREQ:** search frequency.

**H START:** search start time on that frequency.

**H END:** search end time on that frequency.

**TAG:** tag detected.

**DEGREES:** degrees from the direction in which the tag is detected (if known).

**NEG.:** negative; if no tag is detected during that period and at that frequency, mark the square with an X.

**CTx Tag Set-Up**

**Deployment Date:** 15-mar-2023  
**Project Duration (months):** 12  
**Battery:** Ag376  
**Battery days:** 104  
**Pulse Width (ms):** 10  
**Pulse Rate (bpm):** 30  
**NOMINAL Weight Range (g):** 0.9-1.0

**INSTRUCTIONS**

1. Deployment Date (dd/mm/yy) is the date you intend to fit the tags to birds.
2. Project Duration, Battery, Pulse Width, and Pulse Rate can be chosen from the lists.
3. Active days are "1" in the box to the left of the date. An empty box indicates the tag is inactive. Each active period should be at least 2 consecutive days.

If any battery capacity remains at the time of the last active day, the transmitter will transmit every day thereafter until the battery is exhausted.

Note: The dates underlined in yellow are the days on which the Ctx devices are expected to emit a signal

### Calendar

Mon		27-mar-2023	24-abr-2023	22-may-2023	19-jun-2023	17-jul-2023	14-ago-2023	11-sep-2023	09-oct-2023	06-nov-2023	04-dic-2023	01-ene-2024	<u>1</u> 29-ene-2024	<u>1</u> 26-feb-2024
Tue		28-mar-2023	25-abr-2023	23-may-2023	20-jun-2023	18-jul-2023	15-ago-2023	12-sep-2023	<u>1</u> 10-oct-2023	07-nov-2023	05-dic-2023	02-ene-2024	<u>1</u> 30-ene-2024	<u>1</u> 27-feb-2024
Wed		<u>1</u> 29-mar-2023	<u>1</u> 26-abr-2023	<u>1</u> 24-may-2023	<u>1</u> 21-jun-2023	<u>1</u> 19-jul-2023	16-ago-2023	<u>1</u> 13-sep-2023	<u>1</u> 11-oct-2023	<u>1</u> 08-nov-2023	06-dic-2023	<u>1</u> 03-ene-2024	<u>1</u> 31-ene-2024	<u>1</u> 28-feb-2024
Thu		<u>1</u> 30-mar-2023	<u>1</u> 27-abr-2023	<u>1</u> 25-may-2023	<u>1</u> 22-jun-2023	<u>1</u> 20-jul-2023	17-ago-2023	<u>1</u> 14-sep-2023	12-oct-2023	<u>1</u> 09-nov-2023	07-dic-2023	<u>1</u> 04-ene-2024	<u>1</u> 01-feb-2024	<u>1</u> 29-feb-2024
Fri		31-mar-2023	28-abr-2023	26-may-2023	23-jun-2023	21-jul-2023	18-ago-2023	15-sep-2023	13-oct-2023	10-nov-2023	08-dic-2023	05-ene-2024	<u>1</u> 02-feb-2024	<u>1</u> 01-mar-2024
Sat		01-abr-2023	29-abr-2023	27-may-2023	24-jun-2023	22-jul-2023	19-ago-2023	16-sep-2023	14-oct-2023	11-nov-2023	09-dic-2023	06-ene-2024	03-feb-2024	02-mar-2024
Sun		02-abr-2023	30-abr-2023	28-may-2023	25-jun-2023	23-jul-2023	20-ago-2023	17-sep-2023	15-oct-2023	12-nov-2023	10-dic-2023	07-ene-2024	04-feb-2024	03-mar-2024
Mon		03-abr-2023	01-may-2023	29-may-2023	26-jun-2023	24-jul-2023	21-ago-2023	18-sep-2023	16-oct-2023	13-nov-2023	11-dic-2023	<u>1</u> 08-ene-2024	<u>1</u> 05-feb-2024	<u>1</u> 04-mar-2024
Tue		04-abr-2023	02-may-2023	30-may-2023	27-jun-2023	25-jul-2023	22-ago-2023	19-sep-2023	17-oct-2023	14-nov-2023	<u>1</u> 12-dic-2023	<u>1</u> 09-ene-2024	<u>1</u> 06-feb-2024	<u>1</u> 05-mar-2024
Wed		<u>1</u> 05-abr-2023	<u>1</u> 03-may-2023	<u>1</u> 31-may-2023	<u>1</u> 28-jun-2023	26-jul-2023	23-ago-2023	20-sep-2023	18-oct-2023	15-nov-2023	<u>1</u> 13-dic-2023	<u>1</u> 10-ene-2024	<u>1</u> 07-feb-2024	<u>1</u> 06-mar-2024
Thu		<u>1</u> 06-abr-2023	<u>1</u> 04-may-2023	<u>1</u> 01-jun-2023	<u>1</u> 29-jun-2023	27-jul-2023	24-ago-2023	21-sep-2023	19-oct-2023	16-nov-2023	<u>1</u> 14-dic-2023	<u>1</u> 11-ene-2024	<u>1</u> 08-feb-2024	<u>1</u> 07-mar-2024
Fri		07-abr-2023	05-may-2023	02-jun-2023	30-jun-2023	28-jul-2023	25-ago-2023	22-sep-2023	20-oct-2023	17-nov-2023	15-dic-2023	<u>1</u> 12-ene-2024	<u>1</u> 09-feb-2024	<u>1</u> 08-mar-2024
Sat		08-abr-2023	06-may-2023	03-jun-2023	01-jul-2023	29-jul-2023	26-ago-2023	23-sep-2023	21-oct-2023	18-nov-2023	16-dic-2023	13-ene-2024	10-feb-2024	09-mar-2024
Sun		09-abr-2023	07-may-2023	04-jun-2023	02-jul-2023	30-jul-2023	27-ago-2023	24-sep-2023	22-oct-2023	19-nov-2023	17-dic-2023	14-ene-2024	11-feb-2024	10-mar-2024
Mon		10-abr-2023	08-may-2023	05-jun-2023	03-jul-2023	31-jul-2023	28-ago-2023	25-sep-2023	23-oct-2023	20-nov-2023	18-dic-2023	<u>1</u> 15-ene-2024	<u>1</u> 12-feb-2024	<u>1</u> 11-mar-2024
Tue		11-abr-2023	09-may-2023	06-jun-2023	04-jul-2023	01-ago-2023	29-ago-2023	26-sep-2023	24-oct-2023	21-nov-2023	<u>1</u> 19-dic-2023	<u>1</u> 16-ene-2024	<u>1</u> 13-feb-2024	<u>1</u> 12-mar-2024
Wed	<u>1</u> 15-mar-2023	12-abr-2023	10-may-2023	07-jun-2023	05-jul-2023	02-ago-2023	30-ago-2023	<u>1</u> 27-sep-2023	<u>1</u> 25-oct-2023	22-nov-2023	<u>1</u> 20-dic-2023	<u>1</u> 17-ene-2024	<u>1</u> 14-feb-2024	<u>1</u> 13-mar-2024
Thu	<u>1</u> 16-mar-2023	13-abr-2023	11-may-2023	08-jun-2023	06-jul-2023	03-ago-2023	31-ago-2023	<u>1</u> 28-sep-2023	<u>1</u> 26-oct-2023	23-nov-2023	<u>1</u> 21-dic-2023	<u>1</u> 18-ene-2024	<u>1</u> 15-feb-2024	<u>1</u> 14-mar-2024
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Wed	<u>1</u> 22-mar-2023	<u>1</u> 19-abr-2023	<u>1</u> 17-may-2023	<u>1</u> 14-jun-2023	<u>1</u> 12-jul-2023	09-ago-2023	<u>1</u> 06-sep-2023	04-oct-2023	01-nov-2023	<u>1</u> 29-nov-2023	27-dic-2023	<u>1</u> 24-ene-2024	<u>1</u> 21-feb-2024	20-mar-2024
Thu	<u>1</u> 23-mar-2023	<u>1</u> 20-abr-2023	<u>1</u> 18-may-2023	<u>1</u> 15-jun-2023	<u>1</u> 13-jul-2023	10-ago-2023	<u>1</u> 07-sep-2023	05-oct-2023	02-nov-2023	<u>1</u> 30-nov-2023	28-dic-2023	<u>1</u> 25-ene-2024	<u>1</u> 22-feb-2024	21-mar-2024
Fri	24-mar-2023	21-abr-2023	19-may-2023	16-jun-2023	14-jul-2023	11-ago-2023	08-sep-2023	06-oct-2023	03-nov-2023	01-dic-2023	29-dic-2023	<u>1</u> 26-ene-2024	<u>1</u> 23-feb-2024	
Sat	25-mar-2023	22-abr-2023	20-may-2023	17-jun-2023	15-jul-2023	12-ago-2023	09-sep-2023	07-oct-2023	04-nov-2023	02-dic-2023	30-dic-2023	27-ene-2024	24-feb-2024	
Sun	26-mar-2023	23-abr-2023	21-may-2023	18-jun-2023	16-jul-2023	13-ago-2023	10-sep-2023	08-oct-2023	05-nov-2023	03-dic-2023	31-dic-2023	28-ene-2024	25-feb-2024	



Activation/ Deactivation	Technician	Tag ID	When		Comments
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Activation/ Deactivation	Technician	Tag ID	When		Comments
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INSTRUCTIONS FOR DATA COLLECTION

**ACTIVATION / DEACTIVATION:** indicate if the tag has been activated (A) or deactivated (D).

**TECHNICIAN:** name of the workers.

**TAG ID:** write down the tag code.

**DATE:** write down the date on which the activation or deactivation is carried out.

**TIME:** write down the time the tag is activated.

**COMMENTS:** write down if there are any problems.

## APÉNDICE 3.1. METHODS FOR THE TRANSPORTATION AND RELEASE OF BIRDS IN REINTRODUCTION PROGRAMS: RECOMMENDATIONS FOR THE DUPONT'S LARK

### Introduction

Conservation strategies for endangered species increasingly include reintroduction programs to strengthen or recover their populations (Parker, 2008). These programs are an effective tool for restoring ecosystem functions and processes (Benayas et al., 2009; Seddon et al., 2014). Consequently, over the last 30 years, there has been a significant increase in actions involving the release of individuals from other populations (translocations) or from ex-situ conservation programs (Simón et al., 2012; Ferrer & Morandini, 2018). Many of these events have successfully contributed to the recovery of severely threatened species, such as the Iberian lynx (*Lynx pardinus*; Simón et al., 2012) and the Iberian imperial eagle (*Aquila adalberti*; Muriel et al., 2011; Simón et al., 2012). However, a considerable proportion of translocations conducted in these programs fail to establish viable populations in the long term (Oro et al., 2011; Ebrahimi et al., 2015).

Factors influencing the success of translocations include those related to the immediate post-release period (establishment phase) and those prior to release (capture, transportation, and release). Both periods can induce stress in translocated individuals, reducing their survival rate and therefore affecting the establishment of future populations. The post-release period has been more extensively studied and produces its own set of stressors, including those related to arrival in new environments (Dickens et al., 2010), new social interactions (Tavecchia et al., 2009), local competition (Griffith et al., 1989), and predation (Bennett, 2012; Fischer & Lindenmayer, 2000). On the other hand, the pre-release period and the stressors it generates have not been studied as thoroughly, despite playing a crucial role in these projects.

The capture of wild individuals and their retention during translocation generate stress levels that can vary from moderate to high (Adams et al., 2010; Parker et al., 2011). Prolonged stress can lead to a decreased immune response or behavioural problems due to accumulated effects (McEwen, 1998; Dickens et al., 2010). In this regard, the methodology of capture, maintenance, and release is crucial in the translocation process, as it can exacerbate these issues (Parker et al., 2011), increase founder mortality, and reduce reintroduction viability (Griffith et al., 1989). Unfortunately, these methods are often not subjected to analysis and publication (especially transportation methods), leading to a lack of knowledge about previously employed methods (Seddon et al., 2007; Parker et al., 2011).

In this document, we present a compilation of the main transportation and release methods used in previous actions. To select relevant documents, we conducted a Boolean search (see Gago et al., 2016) on Google Scholar and WOS (Web of Science) between May and June 2022, using a combination of the following keywords: 'small bird', 'transport', 'translocation', 'release', 'passerine', 'reintro-

duction’, and ‘restocking’. Based on these previous studies and current knowledge about the Dupont’s lark (*Chersophilus duponti*), we provide a series of recommendations for transportation and release trials of individuals of this species.

### Maintenance and transportation of individuals

The period of captivity between capture and release in the natural environment often impacts the natural behaviour of individuals to be released (Parker et al., 2011). The restriction of evasive movements can increase the risk of injuries and stress-related problems, although these responses may vary between species/individuals (Mason, 2010) and depending on whether the birds to be translocated are wild or were bred in captivity (Parker et al., 2011). For stress-sensitive species (e.g., *Acanthisitta chloris*), it is especially recommended that these periods be minimized and that the birds be transported and released immediately after capture (Leech et al., 2007). For highly territorial or solitary species (e.g., *Petroica spp.*, *Megalurus (Bowdleria) punctata vealeae*, *Alauda razae*), individualized transport would be preferable to avoid conspecific aggression (Lovegrove & Veitch, 1994; Parker, 2002; Brooke et al., 2020), while gregarious species could be transported together (Clarke et al., 2002; Jenni et al., 2014; Delgado et al., 2016).

In addition to the tolerance of the species or individual to be translocated, other factors such as the type of release or logistical limitations can determine the duration of the captivity period. In this case, the design of the enclosure where the birds will be kept in captivity (between capture and release) should take into account the balance between biological requirements and practical limitations and replicate wild conditions as much as possible (Swaigood, 2010). Large enclosures are generally preferable, unless the period between capture and translocation is very short (Sherwin, 2004; Gebhardt-Henrich & Steiger, 2006) or natural movement needs to be limited to reduce stress or potential injuries. In some species (e.g., *Sturnus vulgaris*), the shape of the enclosure also appears to be important as it influences stereotypic behaviour (Asher et al., 2009).

If the captivity period for the individuals to be translocated is very short (a few hours), they can be transported in the same enclosures where they were placed after capture (Withers et al., 2019). On the other hand, if the period is more prolonged (> 1 day), it is recommended that the individuals be moved to larger enclosures first and then transferred to smaller ones for transportation. The main goal is to minimize enclosure changes (e.g., box, bag), thereby reducing novelty and unpredictability. According to some authors, for small birds (passerines), calico bags (cotton bags) can be used for transport if the journey is a few hours long (Bennett, 2012, Brooke et al., 2020; Table 1 & Figure 1), a small transport box if the period is around one day (Withers et al., 2019; Mitchell et al., 2022), or a larger aviary if the period extends to several days (Leech et al., 2007; Richardson et al., 2015; Table 1 & Figure 1).





**Figure 1.** Images of transportation (individual bags suspended inside ventilated boxes) and release (“hard” and simultaneous) of several Razo larks (*Alauda razae*) on Santa Luzia Island (Source: Brooke et al., 2020).

During transportation from capture sites to release sites, birds are exposed to cumulative effects from changes in temperature or humidity, as well as disturbances caused by unexpected noises, impacts, vibrations, and lights (Dickens et al., 2009). To this, we must add the effects of novelty and unpredictability in captive animals (Weiss, 1968), and so these disruptions should be minimized as much as possible. Therefore, during transportation, the animals should be placed in the quietest and most protected locations from temperature changes and lights, and the duration of the journey should be reduced whenever feasible (Parker et al., 2011). Some authors have highlighted the negative effect (stress) of the duration of captivity compared to the method of containment used (Groombridge et al., 2004). However, when extended movements are unavoidable, several techniques can improve success, such as veterinary support and individualized transport of the birds to minimize generated stress (Leech et al., 2007; Reynolds et al., 2008; Bennett, 2012; Brooke et al., 2020).

### Bird release

The release process, like transportation, involves a series of factors that can significantly influence the future success of reintroductions. On one hand, demographic factors, such as the appropriate number of individuals to be translocated, must be considered, and on the other hand, the manner in which these releases are carried out (e.g., hard vs. soft release, see below) (Parker et al., 2011).

For gregarious species, the simultaneous release of individuals can reduce vulnerability to predation (due to antipredator strategies), favouring the survival of released birds (Matson et al., 2004). Previous experiences with socially behaving birds have shown that releasing groups of 10 to 30 individuals favoured the success of reintroductions (Ewen et al., 2001). In contrast, in less social species, survival and reproduction may not be affected by the number of individuals released, so populations can potentially establish with small numbers (Parker et al., 2011). Some successful examples

of such translocations are those conducted in the Seychelles islands with two species of passerines (*Copsychus sechellarum* and *Petroica traversi*), where reintroductions were successful with only five translocated individuals (Lopez-Scepulcre et al., 2008), although this may not be suitable for genetic reasons. The time of year when releases are conducted can also be a relevant factor, as shown by some previous studies where releases carried out during periods with better weather conditions and greater resource availability achieved higher individual survival rates (Tavecchia et al., 2009).

The methodology used in releases has been identified as one of the most determining factors in the survival of reintroduced birds (Davidson et al., 1997; Clarke et al., 2002; Franceschini et al., 2008; Richardson et al., 2015). “Soft releases” refer to those in which individuals are kept captive for a period at the release site to improve their acclimation to the new environment. A variation of this method is “delayed release”, where individuals are kept captive for a variable period between capture and release. This methodology can be beneficial for some species or situations; for example, 1) when there are long distances between the source and recipient populations, 2) when individuals from captive breeding programs are released and they are accustomed to captivity (Mitchell et al., 2011), or 3) when a quarantine of individuals is advised to avoid potential disease spread (Parker et al., 2011). Lastly, “hard releases” are those conducted directly into the natural environment, without any period of captivity between capture and release.

Traditionally, soft releases have been recommended as the most appropriate method, although these recommendations lack scientific consensus (Wanless et al., 2002; Teixeira et al., 2007), and their success may vary among different animal groups (Resende et al., 2021). Soft release can facilitate the acclimatization of a captive-bred animal to the natural environment and reduce stress. However, the same waiting period (acclimatization) will likely increase cumulative stress in animals captured from the wild, thereby raising the chances of traumatic injuries (Dickens et al., 2009). Additionally, soft reintroductions are often much more costly as a result of the complex infrastructure required for maintaining the animals, while at the same time, they do not offer greater guarantees of success than hard releases (Swaisgood, 2010). Several studies recommend rapid capture and hard release as the most suitable procedure for small territorial insectivorous birds (Lovegrove & Veitch, 1994; Lovegrove, 1996).

Soft release can have a negative effect on long-term survival, although this effect may not be observed in the first weeks after release (Richardson et al., 2015). Comparative studies of both methods show significant differences in the survival (higher in hard releases) of individuals released through both systems (Richardson et al., 2015; Table 1), indicating that the idea of benefits (opportunity to adapt to the new environment) from soft releases to animals from the wild may be misguided (Davidson et al., 1997; Clarke et al., 2002; Franceschini et al., 2008; Richardson et al., 2015).

Finally, several complementary methods have been described to increase post-release survival, such as supplementary feeding, providing resting and breeding sites, or using attraction signals (electronic calls) to minimize dispersal (Miskelly et al., 2010; Bradley et al., 2011).

**Table 1.** Compilation of recent publications on the transport and release of birds in the context of bird reintroduction projects.

‘Origin’ refers to the source of translocated individuals: from the wild, captive breeding, or rearing from eggs collected from nests in the field (‘Captive rearing’). ‘Mode’ indicates the method in which individuals were transported: individually or multiple individuals together. ‘Feeding’ refers to whether supplementary feeding was provided between capture and release. ‘Time’ is the period of captivity between capture and release. ‘Type of release’ details the method used to reintroduce the individuals; soft release, hard release, aviary (when delayed release was employed); as well as the period (days) the birds remained captive. ‘Survival’ indicates the percentage of individuals estimated to have survived at least 8 months after release.

Referencia	Año	Especie	Origen	Transporte	Modo	Alimentación	Tiempo	Tipo de liberación	Supervivencia (%)
Leech et al. 2007	2003	<i>Acanthisitta chloris</i>	Medio natural	Caja de madera	Individual	SI	5 días	Aviario 5 días + suelta dura	73
Fountain et al. 2016	2006-2010	<i>Emberiza cirulus</i>	Captive rearing	Boxes	-	Yes	2.5 h	Soft. Chicken breeding and release	25.3
Richardson et al. 2015	2007	<i>Notiomystis cincta</i>	Natural environment	Boxes	Separated by sex and age	Yes	1.25 h	Aviary 9-14 days + soft release	4-77 (soft/ hard)
Withers et al. 2019	2008, 2010	<i>Acanthisitta chloris granti</i>	Natural environment	Wooden boxes	Individual	Yes	< 5 h	Hard	22
Bennett 2012	2009	<i>Climacteris picumnus</i>	Natural environment	Cotton bags in dark, ventilated box	Individual	No	5.92 h	Hard	15
Jenni et al. 2014	2009	<i>Perdix perdix</i>	Captive rearing	-	Groups	Yes	9-33 h	Soft. Chicken breeding and release	-
Delgado et al. 2016	2010-2012	<i>Fringilla teydea polatzeki</i>	Captive breeding	-	Groups	Yes	-	Soft (14 days)	77
Cumming et al. 2021	2015	<i>Alopochen aegyptiacus</i>	Natural environment	Cardboard boxes in opaque cabin	-	-	-	Soft (14 days)	100
Mitchell et al. 2022	2018	<i>Stipiturus mallee</i>	Natural environment	Small, ventilated boxes	Alone or in pairs	Yes	24.5 h	Hard	0
Brooke et al. 2020	2018, 2019	<i>Alauda razae</i>	Natural environment	Cotton bags	Individual	No	15 h	Hard	-

## Considerations for the transport and release of Dupont's lark individuals

The Dupont's lark is a steppe bird that is typically territorial (Pérez-Granados et al., 2016). Males defend their territories throughout the annual cycle (Suárez, 2010), showing a clear preference for patches with approximately 30% vegetation cover and a high percentage of bare ground (Tellería et al., 1988; Seoane et al., 2006; Suárez, 2010). The species displays highly cryptic plumage, is extremely elusive, and is reluctant to fly even in the presence of humans (Tella et al., 2005; Vögeli et al., 2008). Most interactions with the species are auditory, and sightings are challenging to make.

Its distribution is restricted to Spain and northern Africa (Suárez, 2010). The Spanish population, and therefore the European population, decreased by 41% between 2004 and 2015 (Gómez-Catasús et al., 2018), now consisting of approximately 1,300-2,400 breeding pairs (Suárez, 2010). Consequently, European populations are classified as Vulnerable by the IUCN (BirdLife International, 2020).

Considering the previous experiences included in this document, as well as the knowledge of the species' ecology and its delicate conservation status, future translocation methodologies should **minimize the time of captivity between capture and release of individuals** (Leech et al., 2007). As it is a territorial and non-gregarious species, the **individualized transport** of captured birds would be most suitable to avoid conspecific aggression (Lovegrove & Veitch, 1994; Parker, 2002; Brooke et al., 2020). Ideally, translocations should be carried out in a short period (<10 hours), and the **design of the transport enclosure** should **limit natural movement to reduce stress or injuries** (Sherwin, 2004; Gebhardt-Henrich & Steiger, 2006). In this regard, individuals can be **transported in the same compartments where they are placed after capture** (Withers et al., 2019). The use of **cotton bags placed inside well-ventilated and opaque individual boxes** could be a suitable option, which has already used previously (Bennett, 2012; Brooke et al., 2020).

As it is a non-gregarious species with numerous stable populations consisting of a small number of individuals, survival and reproduction should not be directly affected by the number of individuals released simultaneously. For this reason, and being the first pilot action, **releases could begin with a small number of birds (<30)**.

Finally, following the recommendations of previous studies, we suggest **hard release** as the method to be used for the release of small insectivorous territorial birds (Lovegrove & Veitch, 1994; Lovegrove, 1996). All methodologies employed in future translocations should be evaluated and subject to necessary modifications as the work progresses.



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## APPENDIX 3.2. FEASIBILITY STUDY: POPULATION VIABILITY ANALYSIS

The demographic feasibility of the translocation program has been verified through a population viability analysis (PVA; Traba et al., 2019). The results show that reinforcing the three recipient populations with a reduced number of individuals (6 males, 2-4 females) for 3 years significantly extends the viability of these populations, while the extraction of individuals has no effect on the donor populations.

### Methodology

For the population viability analysis, the stochastic simulation program VORTEX 10.5.6 (Lacy & Pollak, 2014) was used, and individual-based models were run with 1,000 iterations to account for demographic, environmental, and genetic stochasticity. All default program values were kept except for those explained below. To assess the medium- to long-term survival of the metapopulation, the models were projected for 20 years, as established in the IUCN criteria. Individual-based models are based on independent outcomes for each individual's fate, thus including demographic stochasticity (random variation in births and deaths), environmental stochasticity (variations in disease, predation, food availability, climate, natural disasters), and genetic stochasticity (fitness reduction due to inbreeding and loss of genetic variability caused by genetic drift randomness) (Lacy, 2000). All PVAs were designed with a subpopulation level ( $n=100$ ) using the Iberian metapopulation structure (García-Antón et al., 2021). In each iteration, a population was declared extinct when at least one of the two sexes became extinct.

Inbreeding depression was included to introduce evolutionary processes into the models. The default value of 6.29 was used in inbreeding depression, as suggested by Lacy and Pollak (2014), as it represents the combined effect of inbreeding on fertility and first-year survival (O'Grady et al., 2006). The correlation in environmental variability between populations was corrected with an intermediate value of 50% (Suárez & Carriles, 2010). The correlation between reproduction and survival also retained its default value of 50%. As in the study by Suárez and Carriles (2010), in each region a catastrophe was included with a frequency of 5% in years when 5% of females did not reproduce, and survival was less than 5%.

The PVA was carried out in two steps. First, we built a base model considering the most plausible value for each population parameter in relation to the currently available information (shown in bold in Table 1). Next, we conducted a sensitivity analysis on the base model (without translocations) to assess the effect of uncertainty and variability on our reference projections. For this purpose, we progressively varied the value of a specific parameter while keeping all others at their base value. We successively varied the values of (1) productivity, (2) number of breeding females, (3) male mortality, (4) female mortality, (5) juvenile mortality, and (6) dispersal survival. The evaluated ranges of the parameters are indicated in Table 1. We performed 1,000 iterations for each scenario, and then summarized the variation in the projected extinction probability at 20 years for each level of sensitivity. Second, we simulated the translocation process to evaluate its effect on both donor and recipient populations. We added the movement of different numbers of males and females to the base model and different levels of apparent survival immediately after translocation (to represent the possibility of mortality and/or dispersal away from the release site). We assessed the effects of these variations on the mean time to extinction of donor and translocated populations, and on the total suitable habitat area.

**Table 1.** The selected parameters for sensitivity analysis with their respective values. The base model is shown in bold.

Parameter	Sensitivity analysis
Productivity	1.2, 1.3, 1.14, 1.5, 1.6, 1.7, 1.8, 1.9
Breeding females	100, 95, 90, 85, 80, 75, 70
Male mortality	0, 10, 20, 30, 40, 50, 52, 60, 70
Female mortality	40, 50, 60, 69, 80, 90
Juvenile mortality	60, 62.5, 65, 67.5, 69, 70, 72.5, 75
Dispersal survival	10, 20, 30, 40, 50, 60, 70, 80, 90, 100

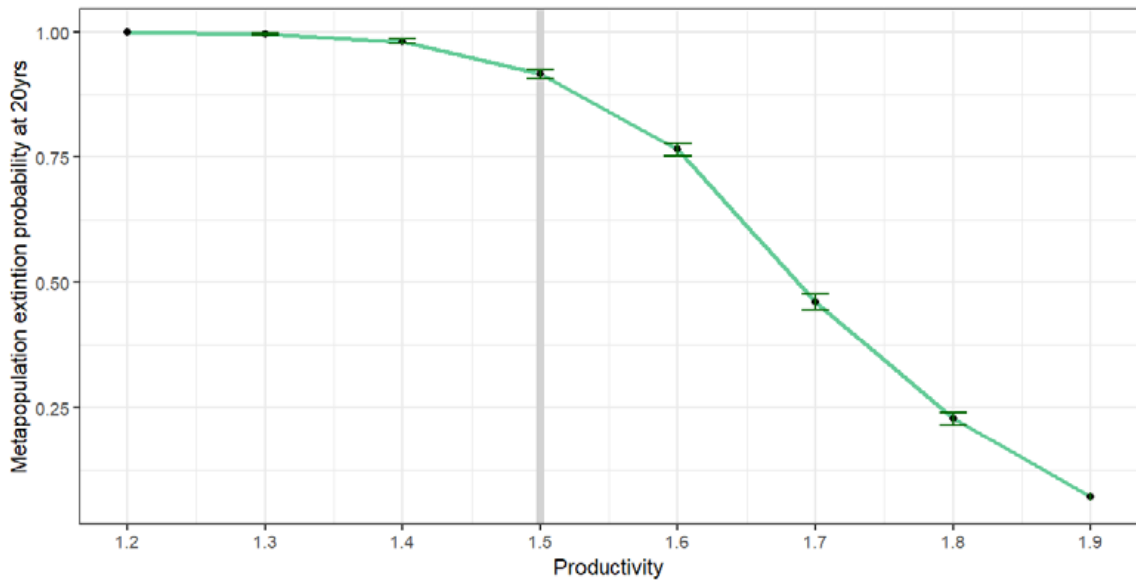
## Results

Below are the graphs showing the results obtained from the various PVA analyses. In each graph, the bars represent the standard error over 1,000 iterations, and - in the sensitivity analysis - the grey bar indicates the parameter value for the base model.

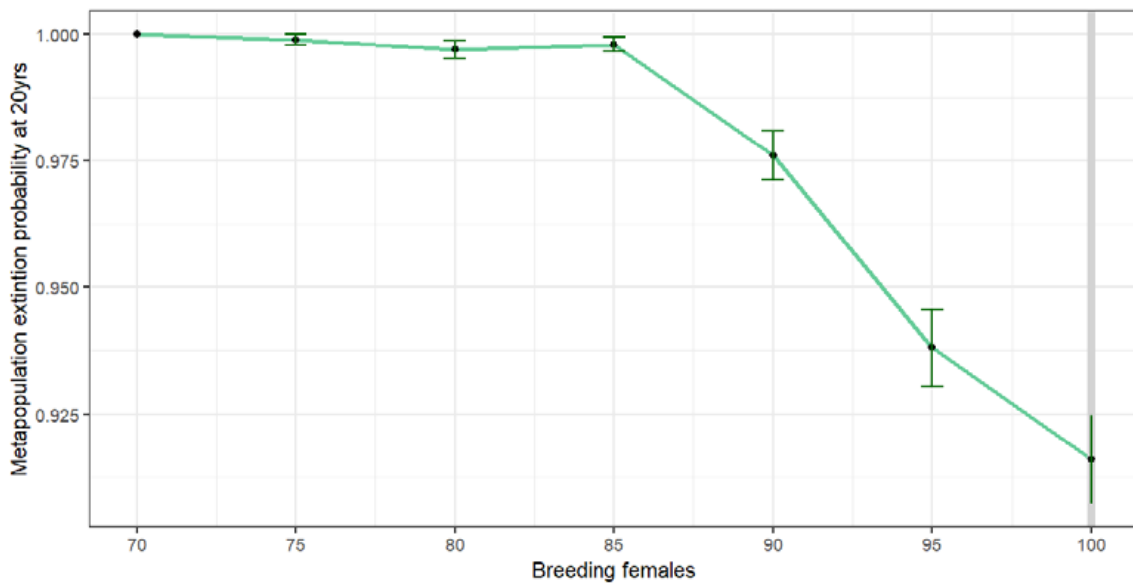
The sensitivity analysis indicates that reproduction is the key stage for conservation. Increasing productivity or the survival of juveniles and females has the most significant impact on population viability (an increase in productivity from 1.5 to 1.7, a reduction in female mortality from 69% to 60%, or a reduction in juvenile mortality from 69% to 65% reduces the probability of extinction by more than 50%; Fig. 1-3).

The simulation of the translocation process indicates that the removal of individuals from donor populations in Parameras de Molina (Molina de Aragón), Altos de Barahona, or Layna did not change the expected mean time to extinction of those populations (Fig. 7). On the contrary, translocated populations are expected to persist for a decade if at least 8-10 individuals (6M/2-4F) are released (Fig. 8). Immediate dispersal or mortality after release can reduce this mean time to extinction by up to 50% (Fig. 9). Finally, as expected, the translocations planned in this project do not significantly change the probability of extinction at the metapopulation level (Fig. 10 and 11).

Based on these results, a translocation of 8-10 individuals per year, with an equal sex ratio whenever possible, provides a reasonable chance of success with little risk to the selected source populations (Section 3.2). Post-release survival (including dispersal) is a key factor for success and presents uncertainty when considering the risk of birds returning to the source populations, especially given the challenges in determining the age of released individuals (Section 4.1). Therefore, in case of insufficient survival, it might be possible to modify the protocol to adopt additional measures that minimize dispersal and promote site fidelity after release, followed by specific monitoring (Section 3.5).

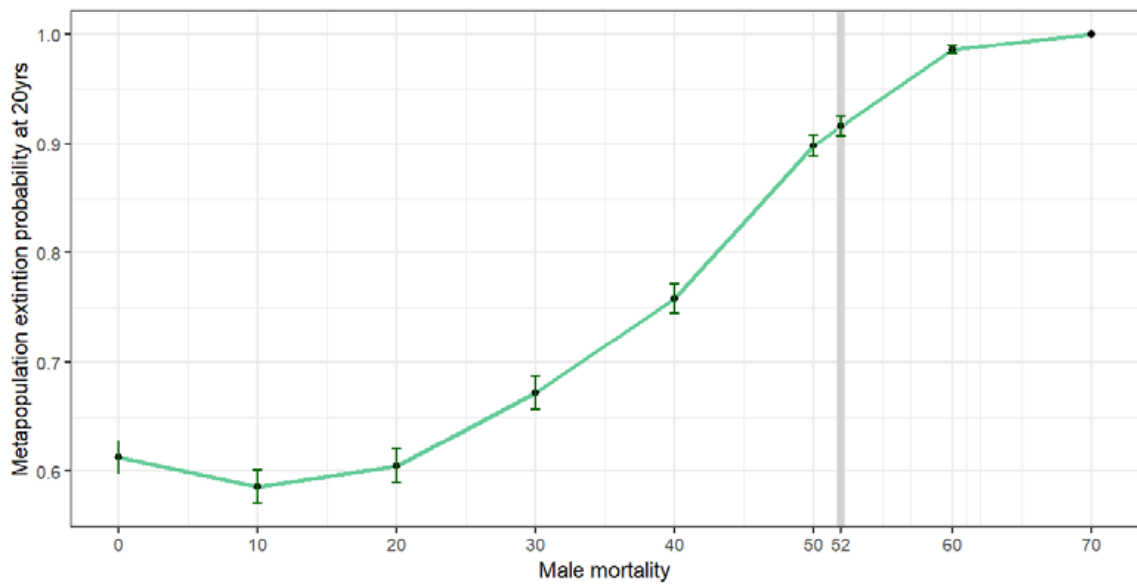


**Figure 1.** The x-axis represents the value of productivity (fledglings per female and brood) introduced in the sensitivity analysis. The y-axis indicates the probability of population extinction at 20 years. The grey bar represents the base model.

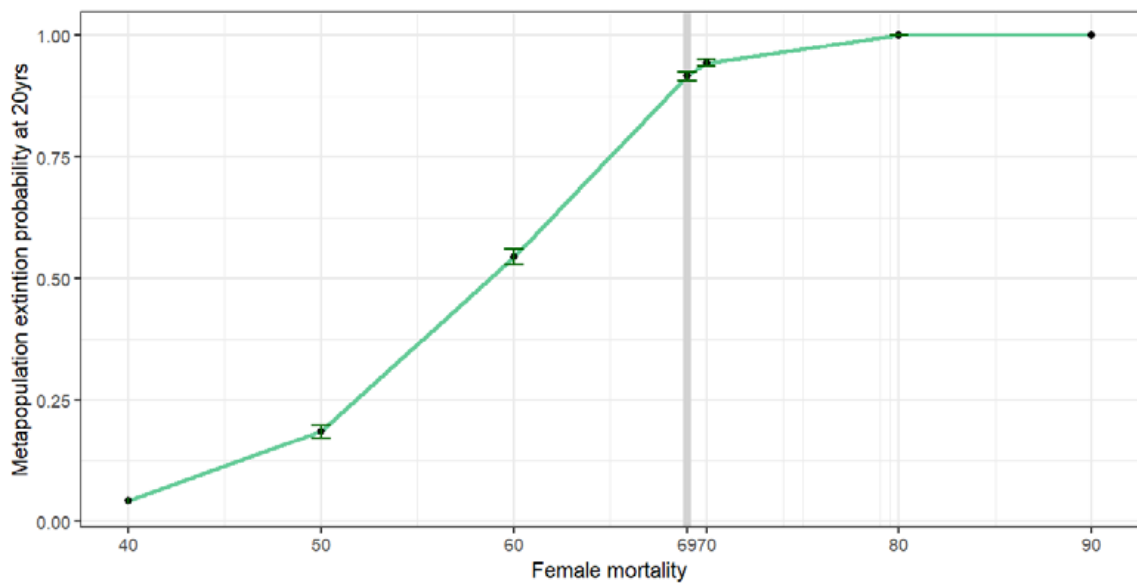


**Figure 2.** The x-axis represents the percentage of reproducing females (each year) introduced in the sensitivity analysis. The y-axis indicates the probability of population extinction at 20 years. The grey bar represents the base model.

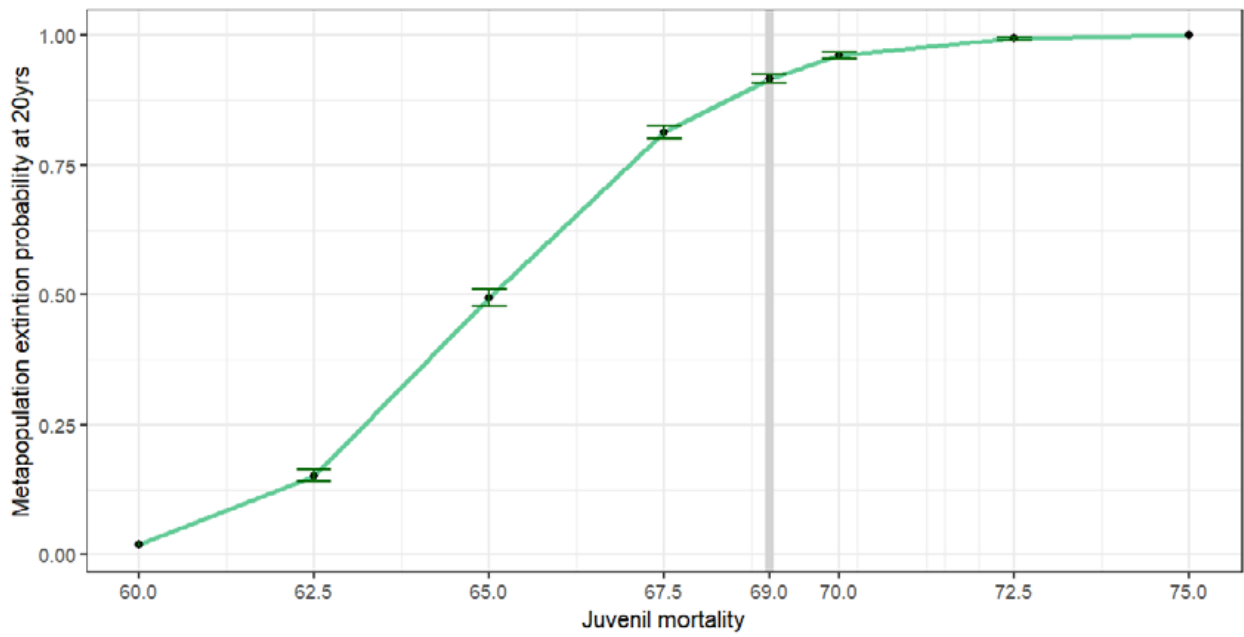




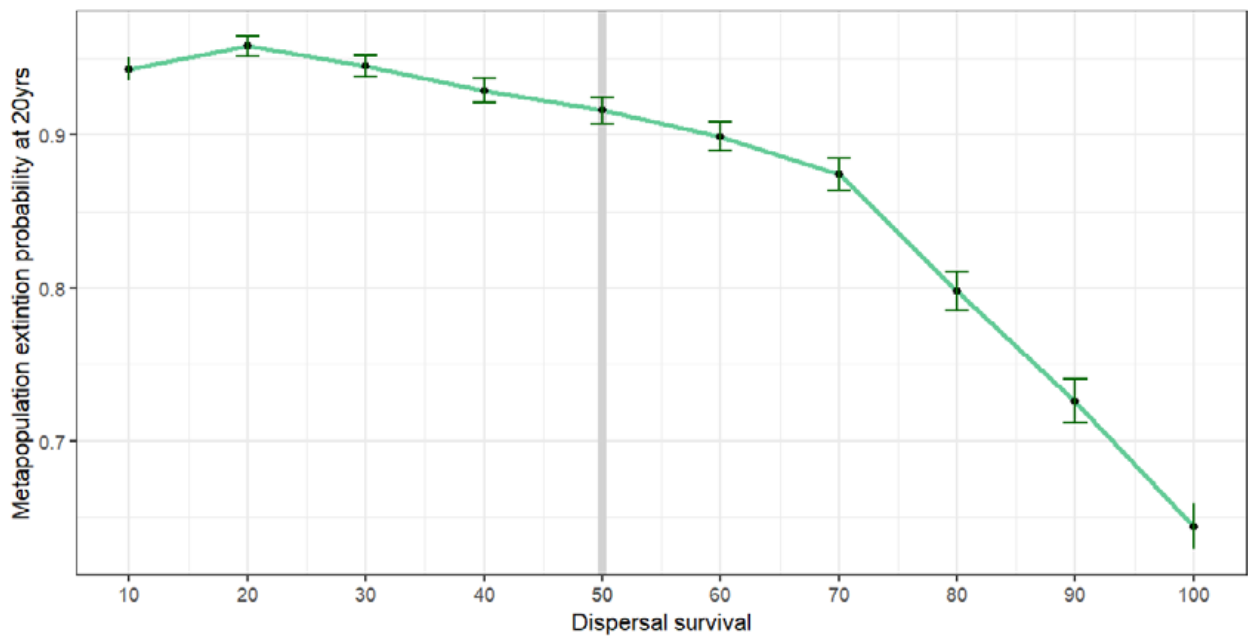
**Figure 3.** The x-axis represents the percentage of male mortality introduced in the sensitivity analysis. The y-axis indicates the probability of population extinction at 20 years. The grey bar represents the base model.



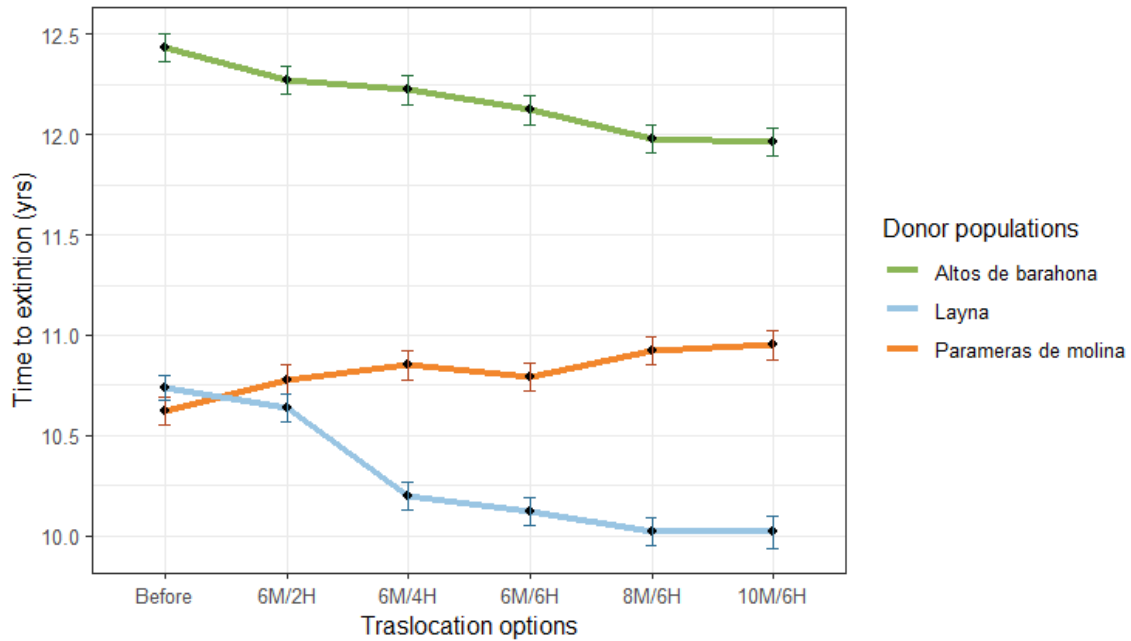
**Figure 4.** The x-axis represents the percentage of female mortality introduced in the sensitivity analysis. The y-axis indicates the probability of population extinction at 20 years. The grey bar represents the base model.



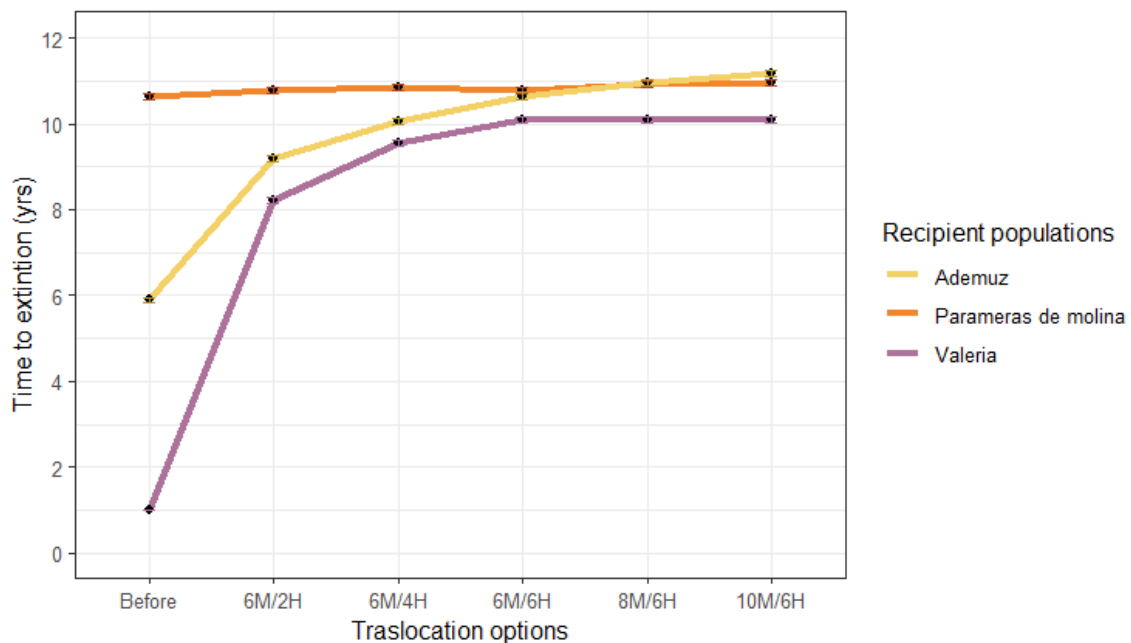
**Figure 5.** The x-axis represents the percentage of juvenile mortality introduced in the sensitivity analysis. The y-axis indicates the probability of population extinction at 20 years. The grey bar represents the base model.



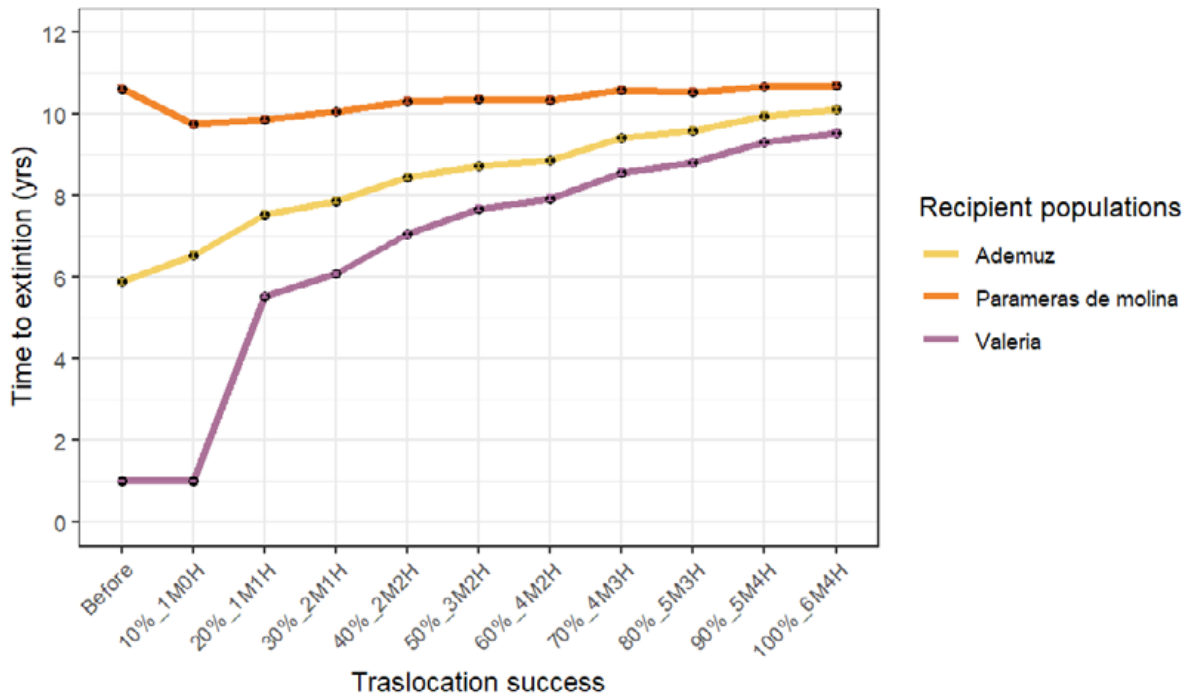
**Figure 6.** The x-axis represents the percentage of juvenile survival in dispersal introduced in the sensitivity analysis. The y-axis indicates the probability of population extinction at 20 years. The grey bar represents the base model.



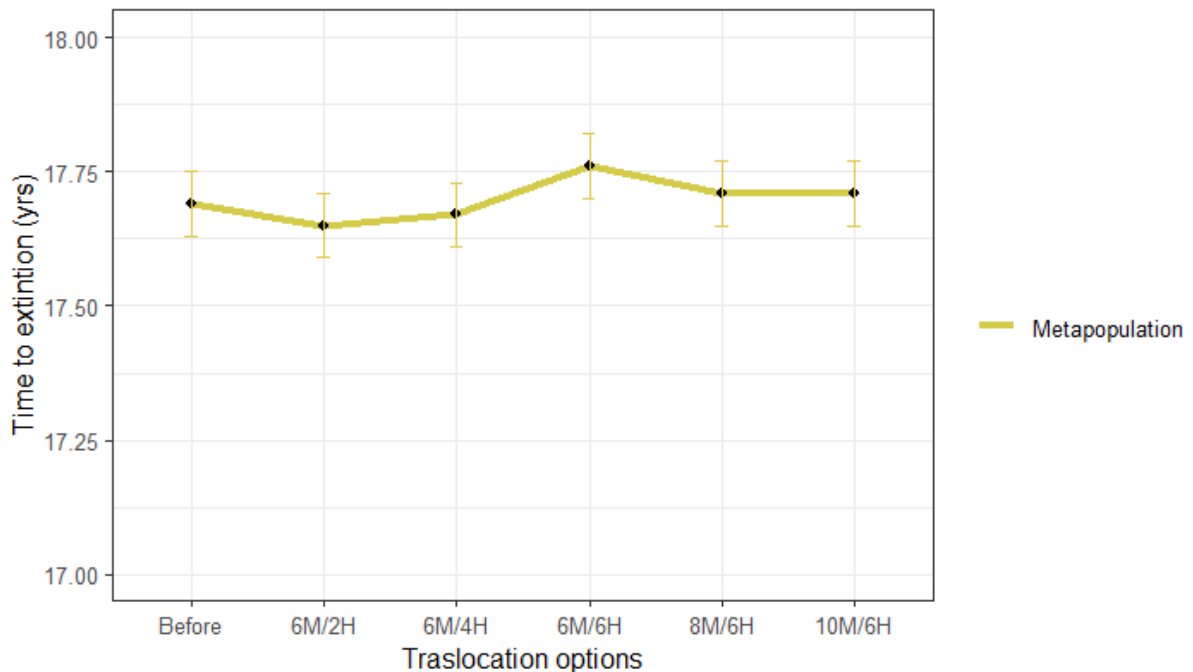
**Figure 7.** The x-axis shows the different translocation combinations, changing the number of males and/or females translocated in each case. The y-axis indicates the average time to extinction for each of the donor populations (over 1,000 iterations).



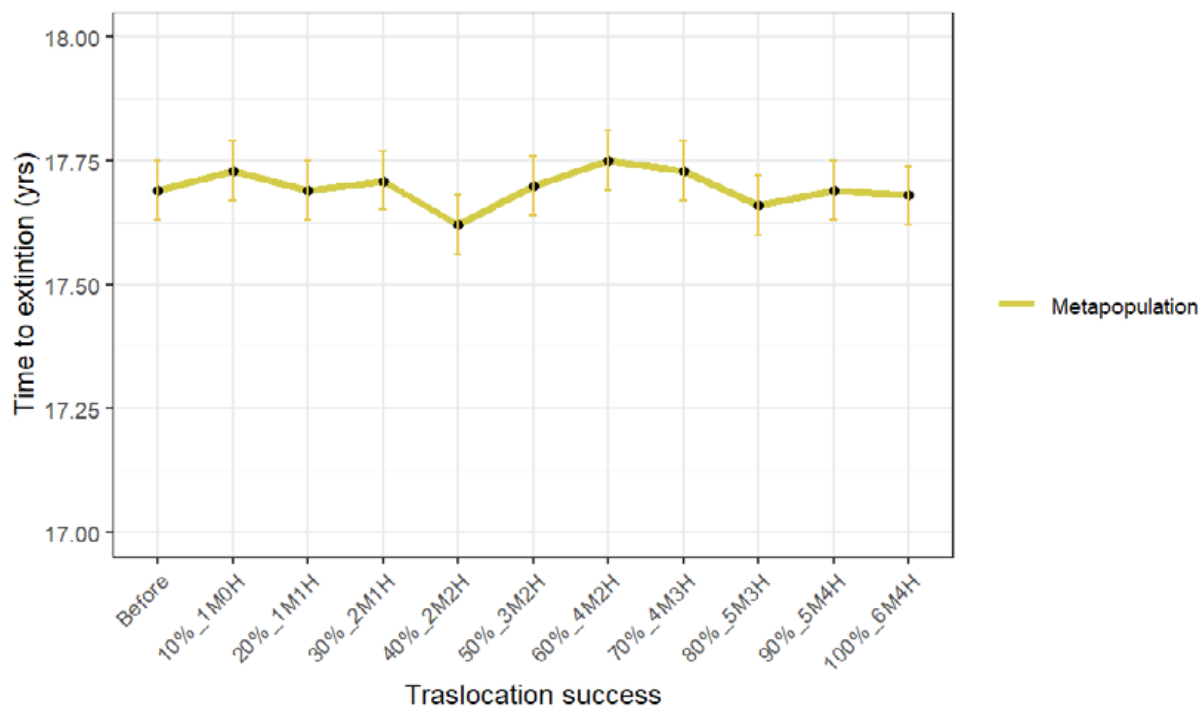
**Figure 8.** The x-axis shows the different translocation combinations, changing the number of males and/or females translocated in each case. The y-axis indicates the average time to extinction for each of the recipient populations (over 1,000 iterations).



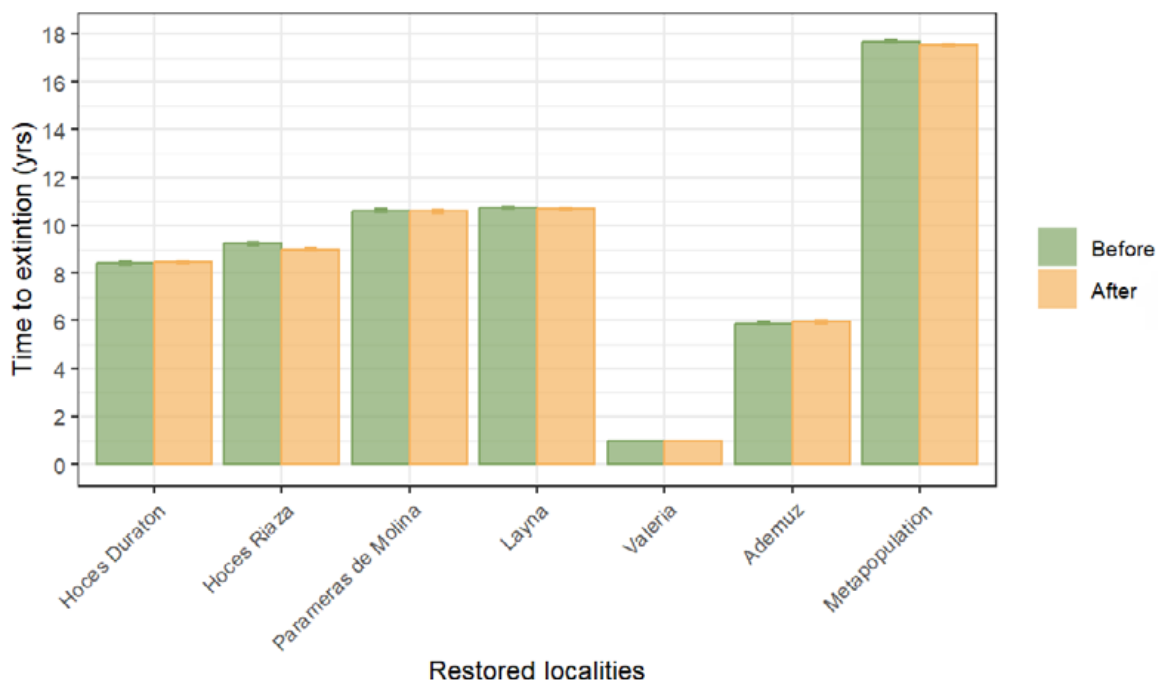
**Figure 9.** The x-axis shows the different success rates of translocation, changing the proportion of individuals surviving the immediate post-release phase (from a base translocation of 6M/4F). The y-axis indicates the average time to extinction for each of the recipient populations (over 1,000 iterations).



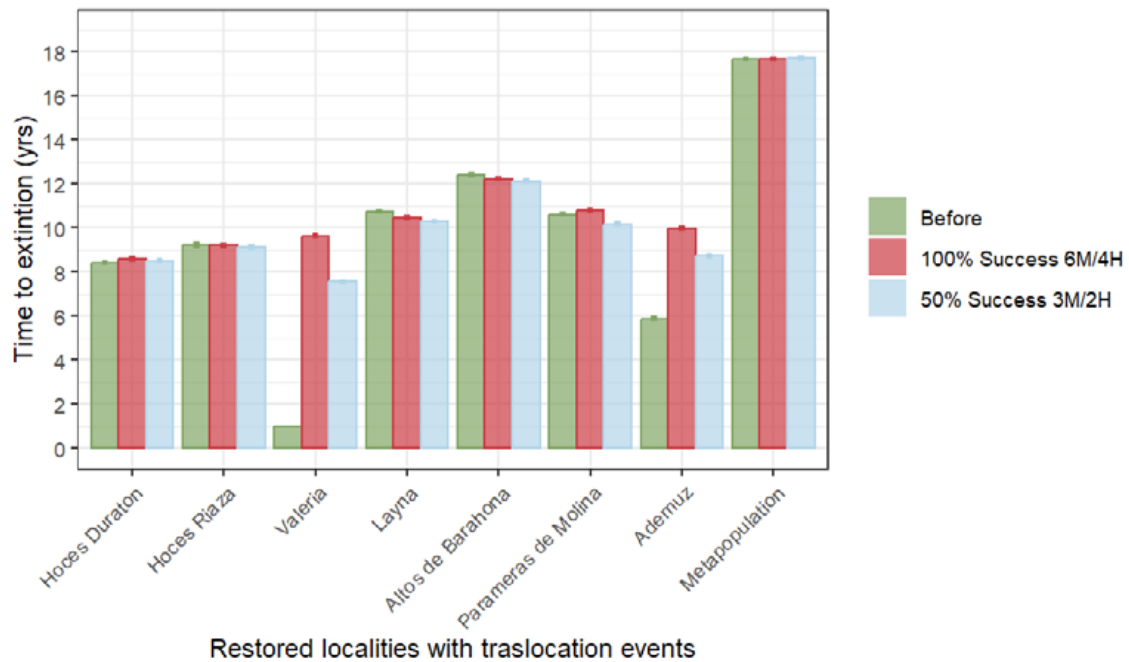
**Figure 10.** The x-axis shows the different translocation combinations, changing the number of males and/or females translocated in each case. The y-axis indicates the average time to metapopulation extinction (over 1,000 iterations).



**Figure 11.** The x-axis shows the different success rates of translocation, changing the proportion of individuals surviving the immediate post-release phase (from a base translocation of 6M/4F). The y-axis indicates the average time to metapopulation extinction (over 1,000 iterations).



**Figure 12.** The x-axis shows the populations that have increased their suitable habitat area. The y-axis indicates the average time to extinction for each population (over 1,000 iterations).



**Figure 13.** On the x-axis, populations that have increased their suitable habitat area (all of them) and have also undergone an individual translocation program (Paramera de Molina, Layna, Altos de Barahona, Valeria, and Ademuz). The y-axis shows the average time to extinction.

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## APPENDIX 3.3. FEASIBILITY STUDY: DISEASE RISK ANALYSIS

### Introduction

Wildlife translocations are an essential tool for improving the conservation status of a target species and/or restoring functions or processes in an ecosystem (IUCN, 2013). However, these movements of wildlife increase the risk of disease, for both the translocated populations and the recipient ones. Translocations raise the probability of contact between hosts and new parasites, exposure to infectious and non-infectious agents during transportation, and the effects of stress on the animals (Davidson & Nettles, 1992; Dickens et al., 2010; Kock et al., 2010). In this regard, the International Union for Conservation of Nature (IUCN) recommends conducting a disease risk analysis and intensive monitoring of all translocated animals (IUCN, 2013).

A disease risk analysis (DRA) is a structured and evidence-based process that can assist in decision-making under uncertainty and determine the potential impact of infectious and non-infectious diseases on ecosystems, wildlife, domestic animals, and humans (Jakob-Hoff et al., 2014). The results obtained through a DRA also help stakeholders consider options for disease prevention and risk mitigation in the populations of interest.

For the development of this DRA, the procedures described by Jakob-Hoff et al. (2014) were followed, and the method published by Sainsbury and Vaughan-Higgins (2012), updated by Bobadilla Suarez et al. (2017) and Rideout et al. (2017), were used.

The present DRA was completed in four stages:

1. Problem definition, identification of objectives, focal questions, assumptions, and limitations.
2. Extensive review of all published literature (and available unpublished data) regarding the biology, ecology, and diseases of the Dupont's lark (*Chersophilus duponti*). All resulting information was compiled into a summary list.
3. This summary was reviewed by a team of veterinarians specialized in wildlife and with relevant experience in the project, who provided information and critical input at each phase of the DRA.
4. The results were used to correct and refine the final document and to issue management and disease risk mitigation measures.

The process is summarized in Figure 3.15 and illustrates the structure that this section follows.





**Figure 1.** Phases of the disease risk analysis (DRA) process (Jakob-Hoff et al., 2014).

### Problem description

The justification and background, as well as the source and destination populations for this translocation, have been well presented in previous sections. The objective of this DRA is to develop strategies to mitigate disease risk in the translocation of Dupont’s lark individuals across the Iberian central plateau, using a structured and scientifically informed analysis of the available information. The focus is to identify, assess, and mitigate all significant health risks to the Dupont’s lark and all vertebrate fauna, domestic animals, and human residents at the release sites that may arise from the proposed translocation. The scope of this DRA is limited to a qualitative risk analysis of all relevant literature available on the susceptibility of the Dupont’s lark to infectious and non-infectious diseases. It also includes considerations of the species’ biology and threats and the impact of these hazards on the program’s ultimate objectives. The specific questions of the DRA are:



- What is the disease risk to the Dupont's lark as a result of the identified health hazards associated with the translocation that could jeopardize the survival and well-being of the **translocated animals**? How can this risk be minimized?
- What is the disease risk to the Dupont's lark as a result of the identified health hazards associated with the translocation that could jeopardize the **resident populations of the Dupont's lark**? How can this risk be minimized?
- What is the risk of translocated larks causing/transmitting disease to **wildlife, domestic animals, and humans** present in the destination areas? How can this risk be minimized?

The stakeholders commit to identifying and implementing mitigation measures to reduce any identified risks to acceptable levels before proceeding with the translocation. The acceptable risk level differs for these three identified populations:

- Translocated Dupont's lark individuals: the translocation should result in low to moderate health impacts.
- Wildlife residing in the release areas (including other larks): the translocation should pose a low risk to individual health and negligible population-level impacts on resident fauna.
- Humans and domestic animals: the translocation should pose a negligible risk of disease.

All DRA in wildlife involve a high level of complexity and uncertainty; therefore, transparency is essential throughout the entire process. This includes explicitly stating the assumptions and limitations of the analysis:

- Assumptions
  - The Dupont's lark is susceptible to the full range of diseases documented in the order Passeriformes.
  - The Dupont's lark is susceptible to generalist bird pathogens that have demonstrated a broad range of hosts.
  - Diagnostics tests and drugs that have been scientifically validated for use in birds can be safely utilized in the Dupont's lark.
- Limitations
  - There is a lack of knowledge about the full range of pathogens that may affect the Dupont's lark and their epidemiology.
  - There is no comprehensive research on pathogens and health issues affecting the wild populations at both source and destination.
  - The individual, population, and ecosystem consequences of many of the considered diseases are unknown.
  - The scope of this DRA is limited to considering disease risks associated with translocations from wild population to wild population within the Iberian central plateau region (not applicable to captive breeding programs or long-distance translocations).



Identification of hazards

**Translocation itinerary and description of barriers**

The translocation itinerary is a visual representation of the route taken by translocated animals and illustrates the points at which different hazards may arise. In this case, it represents the movement of Dupont’s lark individuals from the source sub-populations (Guadalajara, Castilla La Mancha) to the destination area (Guadalajara/Cuenca, Castilla-La Mancha; Figures 3.14 and 3.16). Hazards can be infectious or non-infectious and can be categorized based on the stage of the itinerary in which they act (Table 3.3). Identifying which hazards may be acting for a specific translocation itinerary helps to understand the rationale behind certain hazard identifications, consider alternative routes to avoid them, or implement measures to reduce them.

One of the main considerations in a translocation itinerary is whether it crosses ecological or geographical barriers. **Ecological barriers** are characteristics (e.g., physical, behavioural, or reproductive) that prevent interaction between two populations in the absence of geographical barriers. **Geographical barriers** are natural and environmental features that hinder natural movement between populations (e.g., rivers, mountains, or seas). Distinguishing between itineraries that cross these barriers or not is crucial, as empirical evidence indicates that the most significant disease outbreaks associated with translocations have occurred due to hazards in the source that have crossed these barriers (Sainsbury & Vaughan-Higgins, 2012). The proposed itinerary for this translocation implies that the source and destination environments are not separated by these barriers. In the absence of crossing barriers, hazards from the source and destination are not considered separately, as they are the same, and the overall disease risk is reduced. Therefore, for this DRA, we will only consider carrier, transport, population, and zoonotic hazards.



**Figure 2.** Proposed translocation itinerary for the Dupont’s lark (*Chersophilus duponti*) in the present disease risk analysis. The arrow represents the movement of individuals. The identified hazard types are shown in the yellow box.

**Table 1.** Types of hazards and definitions according to Bobadilla Suarez et al. (2017).

Hazard type	Definition
Origin	Infectious agents transported by translocated individuals that are new (foreign) to the destination environment.
Destination	Infectious agents present in the destination environment to which translocated animals have not been exposed (naïve).
Carrier	Commensal organisms that can cause disease when some stressful effect reduces the host's immune competence and alters the host-parasite relationship.
Transport	Hazards that appear during transport and are new to the translocated animals and/or the destination environment.
Population	Infectious and non-infectious agents that can have negative population-level impacts in the destination environment but are not necessarily new to it.
Zoonotic	Infectious agents transported by translocated individuals that can be transmitted and cause disease in humans.

### Sources of information

Both published literature and unpublished veterinary records describing diseases that can affect Passerine species and other Iberian birds were reviewed. The information was used to create a list of hazards that may be relevant in the translocation of the Dupont's lark within the central Iberian plateau. Subsequently, expert veterinarians from the Wildlife Conservation Medicine (WildCoM) research group at the Autonomous University of Barcelona reviewed the preliminary list with informative notes and made necessary corrections on the basis of their knowledge and personal experience. The final list of identified hazards can be seen in Table 3.4.

On the basis of this preliminary list of 37 identified hazards, the experts prioritized the hazards according to the probability of exposure and the magnitude of consequences in case of exposure. For each hazard, the probability of exposure and consequences for the three at-risk populations were assessed as Negligible - Low - Medium - High. On this basis, the following hazards were selected for a detailed risk assessment:

- Ectoparasites
- Coccidia
- Hemoparasites
- *Aspergillus fumigatus*
- Enterobacteria

The complete list and classification of hazards according to experts can be found in Table 3.2. and 3.3. (see section 3.3.) respectively.

### Risk assessment for prioritized hazards

Risk assessments have been carried out for hazards of special concern to the advisory group, on the basis of their frequent detection in larks or other passerines, or their common association with mortality. The risk assessment for each hazard was based on evaluations of exposure and consequences.

In the **exposure assessment**, the probability that translocated larks are exposed and infected with a hazard was determined, and the biological route necessary for the hazard to spread in animals and the destination environment was described. In the **consequence assessment**, the probability and severity of biological, environmental, or economic consequences associated with the entry, establishment, and spread of the hazard were determined. Finally, to **estimate the risk**, the results of the exposure and consequence assessments were combined to qualitatively evaluate the disease risk associated with a hazard (negligible-low-moderate-high). Strategies for managing and mitigating the risk associated with each hazard are detailed and justified in the next section of this document.

### Ectoparasites (mites, ticks, and lice)

#### Type of hazard: carrier

#### Justification for hazard status

The bird ectoparasite community consists of arthropods that live and feed on the host's surface, on the skin, or in the feathers. Ectoparasites in wild Dupont's larks from the proposed destination area for this translocation (Guadalajara) have been recently described (Talabante Ramírez et al., 2019). In that study, 59.7% of individuals were parasitized by at least one arthropod species, belonging to one of three orders: Phthiraptera (lice), Diptera (hipoboscid fly), and Acarina (mites and ticks), with a high diversity of species. More specifically, 42.9% of individuals had feather mites (family Astigmata), and 16.9% had lice (family Philopteridae, genus *Brueelia*). The study did not report any lesions or clinical symptoms in parasitized individuals. While there are no published documents on tick infestation in the Dupont's lark, the present working group has observed the presence of ticks in the species, although the prevalence of these varies widely between regions (unpublished data). There is also information in other closely related species, such as the common lark, present in the translocation area (Talabante Ramírez, 2017).

While most feather mites and lice are highly specific to each bird species, flies and ticks are usually more generalist. Identifying ectoparasites at the species level is likely impossible as most probably belong to undescribed or yet-to-be-named species. There are documented cases of ectoparasites in all families of passerines that have been investigated; for example, the genus *Brueelia* contains several hundred species.

Therefore, identifying and analysing each ectoparasite separately is an impossible and unnecessary task for this DRA.

In general, ectoparasites establish harmless interactions in healthy wild animals, creating a parasite-host balance that normally does not impact individual health or pose a population problem. However, in animals weakened by other pathologies or subjected to stress, the number of ectoparasites can increase rapidly, causing damage to feathers, skin irritation, or blood loss. In these cases, ectoparasites have effects at the individual level, and more rarely can have population effects (in cases of very small populations or under sub-optimal conditions). Likewise, the introduction of a new ectoparasite into a “naïve” population can also have consequences on well-being and conservation. The present translocation does not involve crossing ecological or geographical barriers, so this latter scenario is not considered possible. Considering that the translocation will most likely induce stress in Dupont’s larks, there is a possibility of diseases related to the presence of ectoparasites.

### Exposure assessment

Considering the proportion of Dupont’s larks that had ectoparasites in the destination area (Talabante Ramírez et al., 2019), the probability that individuals are parasitized before being translocated has been assessed as **high**. Since no ecological or geographical barriers are crossed during the translocation, we assume that both the source and destination populations share the same ectoparasites.

The transmission of ectoparasites generally requires physical contact between individuals, for example, between mates or between parents and offspring in the nest (Tompkins et al., 1996; Clayton et al., 2008). Because of the solitary behaviour of the Dupont’s lark and the host specificity of many ectoparasites, the probability of other larks and other wild species in the destination area being exposed to this hazard is **low**. Likewise, the probability of humans or domestic animals being exposed to Dupont’s lark ectoparasites is **low**.

### Consequence assessment

Ectoparasites have the potential to negatively affect individuals (Dik, 2006; Soares et al., 2016) or act as vectors for other parasites (Harbison et al., 2009). Although high levels of parasitisation can have effects at the individual and population level, most studies are focused on captive animals, and there are no descriptions of disease outbreaks and/or mortality in wild birds. In general, the clinical symptoms of ectoparasite infestations result from immunosuppression and/or concurrent disease. Clinical signs in these cases include itching, excessive scratching and grooming, skin irritation, and, in extreme cases, anaemia. Stress during translocation could lead to clinical disease, but the literature suggests that it is a sporadic occurrence.

Therefore, the probability of ectoparasites having an impact on the translocated population of Dupont’s larks is **low**.

As mentioned earlier, low levels of ectoparasites without causing disease are common in many species of wild birds, so the probability of negative impacts on other Dupont's larks and other wild animals in the destination area is **low**.

The impacts on humans or domestic animals are **negligible**.

### Risk estimation

There is a **high** probability that Dupont's larks are infested with ectoparasites, but a **low** probability of exposure and dissemination to wildlife in the destination area and to domestic animals and humans. The probability of negative consequences at individual, population, environmental, public health, and economic levels is **low**.

The overall risk for this hazard is, therefore, **LOW**. The risk is low but not negligible, so measures should be taken to reduce the carrier-type risk.

### Intestinal coccidia

#### Hazard type: carrier

#### Justification of hazard status

Coccidia are intracellular protozoa of the phylum Apicomplexa. They can infect mammals, reptiles, amphibians, and birds, but there are many species of coccidia, each highly specific to a host animal species. These parasites have been detected in almost all orders of birds and are often found at high prevalence in healthy populations, being considered part of their native flora (Schrenzel et al., 2005; Schoener et al., 2013). Prevalence in wild birds varies between 10 and 66%, depending on species, feeding and migratory habits, and age, among other factors (Dolnik et al., 2010; Bandelj et al., 2015). In fact, they are so common that in other translocations of passerines, they have been intentionally conserved (McGill et al., 2010). The coccidia that infect passerines belong to the genus *Isospora* and are excreted in faeces, although species of the genus *Eimeria* and *Caryospora* have also been described.

Although frequently found in healthy animals, coccidia can increase in number and virulence in situations of stress and immunosuppression. Younger individuals often have a higher parasitic load, making them more susceptible to severe clinical conditions. Coccidiosis is a significant cause of disease in captive passerines, usually resulting from poor hygiene, high densities, and stress. In cases where disease associated with intestinal coccidia develops, clinical signs are usually intestinal, while extraintestinal infections are less common but associated with higher mortality. However, the pathogenic effect of coccidia on wild populations has not been extensively investigated. Although there are no specific studies in Dupont's larks, cases of severe intestinal and hepatic coccidiosis have been described in the ciril bunting (*Emberiza cirilus*), a passerine bird, during a translocation project in the United Kingdom (McGill et al., 2010). In that study, the authors concluded that coccidiosis could pose a hazard for the translocation of other passerines and should be considered a significant disease.

## Exposure assessment

To date, there are no published studies on coccidia prevalence in Dupont's larks, so the probability that translocated animals are carriers of coccidia is difficult to assess. The most reliable information available is based on extrapolating the prevalence described in other passerines. Coccidia are transmitted through the ingestion of infective oocysts (faecal-oral route). Therefore, translocated larks could also become infected during transportation, especially if the bags and cages used for transport have previously contained other individuals and are not properly disinfected. In summary, the probability that translocated larks are infected with intestinal coccidia has been assessed as **moderate**.

Infected Dupont's larks released during the translocation will transport intestinal coccidia to the destination area. Coccidia are highly host, tissue, and cell specific (Schrenzel et al., 2005). Although information is limited, it is very likely that coccidia infection in the population of Dupont's larks in the destination area is prevalent. Therefore, the probability of other larks and other wild species in the destination area being exposed to this hazard is **low**. The probability of exposure for domestic animals and humans is **negligible**, based on their taxonomic distance from Dupont's larks.

## Consequence assessment

As mentioned, most cases of coccidiosis in passerines occur in captivity and are associated with stressful stimuli, poor hygiene conditions, concomitant pathologies, etc. (McGill et al., 2010). Clinical signs of coccidiosis include diarrhoea, fever, lack of appetite, weight loss, emaciation, and, in extreme cases, death. Although there are no reported cases of coccidiosis in Dupont's larks, it is expected that the translocation will be a stressful stimulus for the translocated individuals. Therefore, translocated animals infected with coccidia may suffer negative consequences with a **moderate** probability.

For the populations of Dupont's larks or other wildlife in the destination area, the probability of negative consequences derived from coccidia is **low**. For humans and domestic animals, the consequences are **negligible** due to the inability of avian coccidia to infect them.

## Risk estimation

The probability of exposure and infection in translocated Dupont's larks is **moderate**, but it is **low** for the rest of the wildlife and **negligible** for domestic animals and humans. The consequences of coccidiosis can be **moderate** in the translocated population but **low** in Dupont's larks from the destination area and other wildlife.

Therefore, the overall risk for this hazard is **MODERATE**, and measures should be taken to reduce the risk associated with coccidia.

## Hemoparasites

### Hazard type: carrier

#### Justification of Hazard Status

Hematophagous parasites or hemoparasites of the order *Haemosporidia* represent a heterogeneous group of organisms widely distributed worldwide, which are transmitted by vectors and can infect birds, reptiles, amphibians, and mammals (Rivera et al., 2013; van Hemert et al., 2019). Prevalence can vary greatly depending on the habitat and bird species studied (Sehgal, 2015), as well as the host-vector specificity and ecological requirements of the vector (Rivera et al., 2013). A prevalence of 10% has been detected in birds in Spain in the southern part of the Peninsula (Rivera et al., 2013). However, many studies are based on diagnostic methods with low sensitivity (e.g., direct observation in blood smears), and therefore, the prevalence of hemoparasites in wild birds may be underestimated.

*Haemoproteus* species that infect birds are intraerythrocytic parasites transmitted by ceratopogonid midges (*Ceratopogonidae*) (Rivera et al., 2013). These are the most commonly detected hematophagous parasites in passerines, although their potential as a cause of disease in wild bird populations remains unknown. Some species of *Haemoproteus* can be highly pathogenic, causing severe myositis in some birds, although documented cases are rare (Atkinson, 2009a, 2009b).

*Plasmodium* is a group of intracellular parasites transmitted by mosquitoes (*Culicidae*), and they are the causative agents of avian malaria. There are more than 40 species of *Plasmodium* that differ in terms of host, geographic distribution, vectors, and pathogenicity. Various cases of individuals with acute pathogenic infections have been described, but outbreaks affecting several individuals are rare. The disease is primarily associated with birds in captivity or populations of birds encountering the pathogen for the first time, as in the case of vector introductions on remote islands, such as the introduction of *P. relictum* in Hawaii (Atkinson, 2009b, 2009a).

The species of *Leucozytoen* are transmitted by black flies (*Simuliidae*) (van Hemert et al., 2019). It is a pathogen that is order, family, or even species-specific to the host. There are several species, of which only a few are pathogenic, with waterfowl, pigeons, galliformes, birds of prey, and ostriches being the main at-risk groups (Forrester & Greiner, 2009).

*Trypanosoma* can be transmitted by various insects, mainly through vector ingestion (Sehgal, 2015).

Although the impact of hemoparasites at the individual and population level is difficult to discern, there is accumulating evidence of direct and indirect effects of acute and chronic infections. In some species, effects on survival, especially in young animals, and individual reproductive success have been described (Merino et al., 2000; Dadam et al., 2019). Chronic infections may have sublethal, cryptic, or difficult-to-quantify effects on bird populations. Additionally, wild birds are often parasitized by more than one species of hemoparasite and other parasites. As a result, hemoparasites could have additive effects or interact with other agents, compromising the health or behaviour of the animals.



Without causing direct mortality, hemoparasites can increase the susceptibility of their hosts to predation or other diseases. To date, there are no published studies on hemoparasite detection in the Dupont's lark. Overall, there is much uncertainty regarding the level of parasitisation and the impacts of hemoparasite infections on wild bird populations.

### Exposure assessment

There are no specific studies on the prevalence of hemoparasites in Dupont's lark populations, but as these parasites are commonly present in various bird populations on the Iberian Peninsula, and animals can be carriers without showing clinical signs, we must consider the possibility that larks in the source area are infected. Because of the need for vectors for transmission, there is a low likelihood of infection of translocated animals during transportation. Thus, the probability of captured larks being infected with hemoparasites is **moderate**.

The population of Dupont's lark present in the destination area, as well as other birds, may be indirectly exposed to hemoparasites through vectors that have bitten an infected translocated Dupont's lark, contact with faeces, or ingestion of infected vectors. The present translocation does not involve crossing ecological or geographical barriers, so the introduction of a new hemoparasite to the destination population is not considered possible. Because of this indirect transmission, the probability of exposure to Dupont's larks or other wild fauna in the destination area is **low**.

Bird hemoparasite species are not capable of infecting humans or domestic animals, so the probability of exposure in this group is **negligible**.

### Consequence assessment

As mentioned earlier, infections by hemoparasites at low intensities are often asymptomatic. However, in some cases, they have been associated with high mortality, especially in "naïve" populations and animals in captivity. More recently, hemoparasites have been associated with decreases in survival and effects on reproduction in some birds. Clinical manifestations of the disease include lethargy, anorexia, anaemia, and feather abnormalities. The probability of negative consequences in translocated Dupont's larks has been assessed as **low**.

Since the introduction of new hemoparasites is not possible, we consider that the consequences of hemoparasite infection in Dupont's larks or other wild fauna in the destination area are **low**.

**The probability of negative consequences in domestic animals and humans is negligible.**

### Risk estimation

The probability of translocating Dupont's larks carrying hemoparasites is **moderate**. However, the probability of exposure to fauna in the destination area is **low**, and **negligible** for humans and domestic animals. The consequences of hemoparasite infection are **low** for both translocated Dupont's larks and the rest of the fauna in the destination population.

Therefore, the overall risk for this hazard is **LOW**. However, due to the lack of information on hemoparasites in the Dupont's lark, measures should be taken to reduce this hazard, starting with characterizing the presence and prevalence of hemoparasites in this population.

### *Aspergillus fumigatus*

#### Type of hazard: carrier and transport

#### Justification of hazard status

Aspergillosis is a disease caused by filamentous fungi of the genus *Aspergillus*, commonly *A. fumigatus*. These fungi have a worldwide distribution, except for Antarctica, and are ubiquitous in the environment, where they exist in the form of spores (O'Meara & Witter, 1971). A wide variety of birds are susceptible to infections by *A. fumigatus*, which occur when they inhale spores from the environment. The most susceptible bird species are waterfowl (ducks, gulls, shorebirds), followed by Accipitriformes (eagles and hawks) and passerines (Arné et al., 2021). Many birds are likely carriers of *A. fumigatus* spores in their lungs or air sacs without developing disease. If the number of inhaled spores is very high or the bird's immune system is compromised, possibly as a result of stress, individuals can develop clinical disease (Bauck, 1994; Oglesbee, 1997). Aspergillosis is typically a respiratory tract disease.

*Aspergillus fumigatus* presents a wide diversity of strains (Chazalet et al., 1998), and the strains could be different between the proposed source, transportation, and destination areas for this translocation. However, the present translocation does not involve crossing ecological or geographical barriers, so it is considered that the strains are the same. Although there are no previous studies, it is likely that some wild Dupont's larks may be carriers of fungal spores in their lungs and air sacs, or they may become infected during transportation. Considering that translocation is a stressful phenomenon, some disease related to the presence of *A. fumigatus* may occur.

#### Exposure Assessment

*Aspergillus fumigatus* is found in the environment (e.g., soil, decomposing vegetation, agricultural waste) (Chute et al., 1965). Inhalation is considered the primary route of infection in birds. Because of this transmission mechanism and its ubiquity, it is quite likely that there is infection in the captured Dupont's larks for the translocation. Likewise, exposure to *A. fumigatus* can occur at any stage of the translocation, especially during transportation, particularly under unhygienic conditions, high humidity, high temperature, and high densities of individuals. Factors such as humidity, high temperatures, poor ventilation and hygiene, and long-term food storage increase the number of spores in the environment and, therefore, increase the likelihood of a bird developing infection and disease (Beernaert et al., 2010). The probability of infection also increases if the immune system of translocated individuals is compromised by the effects of stress. Therefore, the probability that translocated

Dupont's larks are exposed or carriers of *A. fumigatus* is **moderate**.

Aspergillosis is an infectious but non-contagious disease, as *A. fumigatus* is not transmitted horizontally (between individuals) or vertically (through eggs) (Kearns, 2014). Therefore, the probability of transmission between Dupont's larks or other animals, including humans, is **negligible**.

### Consequence assessment

A high concentration of spores in the environment and a compromised immune system can cause aspergillosis in birds. As mentioned earlier, various factors can increase a bird's risk of developing aspergillosis, such as high animal densities, stress, concomitant pathologies, or certain environmental conditions. The disease can be acute or chronic. Acute disease usually occurs in adult or debilitated animals inhaling a large number of spores. Chronic disease is more common in adult animals under stress or immunosuppression. Symptoms are typically respiratory, including difficulty breathing, weakness, lack of appetite, and emaciation, but they can affect other organs with a variety of clinical signs (Kearns, 2014).

Considering the stress derived from the translocation process, but also the brevity of this stress and the lower susceptibility of passerines, the probability that translocated Dupont's larks develop aspergillosis is **low**. The probability of causing negative consequences for the wild fauna in the destination area, environmental impacts, or health issues for domestic animals and humans due to *A. fumigatus* is **negligible**.

### Risk estimation

The probability of exposure of Dupont's larks to *A. fumigatus* from translocation is **moderate**. However, the negative consequences that this hazard may cause have been assessed as **low**. The probability of transmission to other animals or humans and negative consequences for them is **negligible**.

Therefore, the overall risk for this hazard is **LOW**, and measures should be taken to reduce the risk of *A. fumigatus*.

## Enterobacteria

### Type of hazard: carrier and zoonotic

In this DRA, we consider enteric pathogens to be the enterobacteria (Family Enterobacteriaceae) *Salmonella spp.*, *Yersinia spp.*, *Campylobacter spp.*, and *E. coli*.

### Justification of hazard status

These four enterobacteria are widely distributed worldwide, especially in environments subject to faecal contamination, or contaminated water or food. They can infect a large number of species without necessarily causing disease. Enterobacteria are mainly transmitted through the oro-faecal route and colonize the gastrointestinal system. They are widely distributed in wild bird populations (Gavi-

er-Widén et al., 2012), and although there are no specific studies on the Dupont's lark, it is highly likely that they are susceptible to infection by these pathogens, and some individuals in the populations undergoing translocation may be carriers of enterobacteria.

### *Campylobacter spp.*

Species of the genus *Campylobacter* are distributed worldwide among domestic and wild animals and birds, but in most cases they live as commensals in the oral cavity and intestinal tract mucosa. Wild bird and mammal populations are considered reservoirs of *Campylobacter* (Speck, 2012). The species isolated in wild birds are *Campylobacter coli*, *C. hyointestinalis*, *C. jejuni*, and *C. lari*. Generally, each host species carries a specific strain of *Campylobacter spp.*, indicating that interspecific transmission is rare (Waldenström et al., 2007; Colles et al., 2008). Waldenström et al. (2002) examined 1,794 birds belonging to 107 species from 26 families, and they found an overall *Campylobacter* prevalence of 21.6%, but which varied from 0 to 100%. Certain bird taxa had a high prevalence (e.g., sandpipers, wagtails, pipits, starlings, and thrushes). In the study by Kapperud and Rosef (1983), 540 wild birds were examined, and the overall prevalence was 28.6%. Clinical disease in free-living animals has not been described to date. Zoonotic events related to wild fauna are very rare, but there has been a documented event of *Campylobacter spp.* contamination of milk intended for human consumption through small crows (*Coloeus spp.*) and blue tits (*Parus caeruleus*) (Hudson et al., 1991). Specific studies indicating the prevalence of *Campylobacter spp.* in the Dupont's lark have not been reported, but there are works documenting the prevalence of the bacterium in passerines. Such prevalence ranges from 1 – 67% (Keller et al., 2011; Konicek et al., 2016; González-Acuña & Llanos-Soto, 2020).

### *Escherichia coli*

The vast majority of *E. coli* belong to the normal intestinal flora and are not pathogenic. Although few reports describe disease caused by *E. coli* in wild birds, in Europe, this pathogen may be considered the most prevalent opportunistic enterobacterium in captive birds and has been associated with systemic diseases in birds, usually caused by avian pathogenic *E. coli* (APEC). Vectors (e.g., *Stomoxys calcitrans* and *Musca domestica* flies) can also transmit *E. coli*. Similar strains have been isolated in wild mammals, birds, and livestock, indicating transmission between domestic animals and wildlife or a common environmental source (Speck, 2012). Regarding the Dupont's lark, specific reports do not exist, but there are studies concerning passerines in relation to *E. coli* infection. One of them revealed that the *E. coli* O86:K61 strain was associated with disease and high mortality in wild birds such as chaffinches (*Fringilla coelebs*), greenfinches (*Carduelis chloris*), and Eurasian siskins (*Carduelis spinus*) (Pennycott et al., 1998). Another study in Poland found that the *E. coli* O86 strain could contribute to chick mortality in wild sparrows (*Passer spp.*) (Pawiak et al., 1991). In passerines in Spain, a study revealed that 52.9% of house sparrows (*Passer domesticus*) and 57.1% of starlings (*Sturnus unicolor*) sampled were carriers of some strain of *E. coli*, most of which were non-pathogenic (Sacristán et al., 2014).



### *Salmonella spp.*

*Salmonella* species, especially *S. enterica*, cause infections in various bird species in Europe and worldwide. Outbreaks of mortality have been reported in passerines from several European countries, most associated with the aggregation of multiple individuals at garden bird feeders (Benskin et al., 2009). Clinical signs vary depending on the species and strain of *Salmonella*, the infective dose, and the immune status of the animal (Gaffuri & Holmes, 2012). In passerines, *Salmonella* infection can cause lesions in the crop and oesophagus, but other organs like the liver can also be affected (Refsum et al., 2003). Regarding the Dupont's lark, outbreaks of mortality due to *Salmonella* have been described in the song thrush (*Turdus philomelos*) (Velarde et al., 2012), and a study in the centre of the Iberian Peninsula detected a 5.4% prevalence in house sparrows (*Passer domesticus*) and a 2.7% prevalence in starlings (*Sturnus vulgaris*) (Martín-Maldonado et al., 2020).

### *Yersinia spp.*

Yersiniosis can be mainly caused by two species: *Yersinia pseudotuberculosis* or *Y. enterocolitica* (Bottone, 1999). *Yersinia spp.* strains have been isolated from the intestinal tract or faeces of more than 50 species of European birds and are commonly considered reservoir species. Infections in birds are generally asymptomatic, but disease outbreaks have been described in several species, causing septicaemia and high mortality (Najdenski & Speck, 2012). Although there are no specific studies on the Dupont's lark, infections have been reported in different species of passerines in Europe (Macdonald, 1965; Mair, 1973; Niskanen et al., 2003). Regarding Spain, a recent outbreak of yersiniosis has been described in blackcap warblers (*Sylvia atricapilla*) in the Ebro Delta (Velarde et al., 2021). However, a study conducted in central Spain did not detect *Yersinia* in any samples of pied flycatchers (*Ficedula hypoleuca*), an insectivorous passerine like the Dupont's lark (Ruiz-de-Castañeda et al., 2011).

### *Exposure assessment*

There are no studies on the prevalence of enterobacteria in Dupont's lark populations, but they are very common infectious agents that infect a wide range of species (Daoust & Prescott, 2007). Likewise, exposure to enterobacteria can occur at any stage of translocation, especially during transportation, particularly under unhygienic conditions and high densities of individuals. Therefore, there is a **high** probability that translocated Dupont's larks will be carriers of these bacteria in their intestinal tract.

Stress is a factor that could lead to diseases caused by *Salmonella spp.*, *Campylobacter spp.*, *Yersinia spp.*, and *E. coli* (Gavier-Widén et al., 2012), which can also lead to environmental contamination through the elimination of bacteria from infected animals. Translocation is a stressful process; thus, translocated Dupont's larks carrying enterobacteria may increase the excretion of enterobacteria upon release into the environment of the destination population. The persistence and spread

of enterobacteria in the environment depend on many factors such as temperature and rainfall. However, due to their insectivorous diet and non-gregarious nature with limited interaction among conspecifics (Gómez-Catasús et al., 2016), the probability that Dupont's larks or other wildlife in the destination area will be exposed to enterobacteria through contaminated food from Dupont's larks is **low**. It is not considered possible to introduce new strains of enterobacteria into the destination environment since the present translocation does not cross ecological or geographical barriers.

The probability of exposure in humans and domestic animals is considered **low** as transmission is faeco-oral, i.e., through the consumption of faeces or contaminated food.

### Consequence assessment

As mentioned before, stress is a factor that can contribute to the development of disease by enterobacteria. Translocation is a stressful process; therefore, there is a low but not negligible probability that some translocated Dupont's larks carrying enterobacteria may develop clinical disease with possible mortality during the process.

Although Dupont's larks and the rest of the wildlife in the destination population could become infected with new strains of these bacteria and develop a clinical condition, the probability of this scenario is **low**.

Similarly, in the unlikely event of acquiring infection after contact with an infected Dupont's lark, the probability of humans or domestic animals developing enterobacteria-associated disease is **low**.

### Risk estimation

In summary, the probability of exposure of translocated Dupont's larks to enterobacteria is **high**, but **low** for the destination environment, domestic animals, and humans. The consequences derived from this hazard have been assessed as **low** in the three risk groups considered.

Therefore, the overall risk of this hazard is **LOW** to **MODERATE**, considering the lack of knowledge about prevalence and strains and the zoonotic potential of enterobacteria.

### Management and risk mitigation options

Below, potential methods to reduce the risks associated with different hazards are communicated in a reasoned, referenced, and logically discussed manner.

#### Ectoparasites

1. Perform a thorough clinical examination of the animals to determine their health status and only translocate seemingly healthy animals with low parasite loads. High ectoparasite loads may indicate the presence of other systemic diseases. A detailed examination of the presence and load of ectoparasites can help identify the families or species present in the Dupont's lark population and increase knowledge. The visual inspection procedure is straightforward; however, some ectoparasites may go unnoticed.



2. Use topical antiparasitic treatments (permethrin or fipronil) to prevent high parasite burdens and, therefore, clinical disease. The effectiveness of these compounds is not 100% (Clayton et al., 2008), but if ectoparasites are normal in the species, it would be advisable to retain them in the Dupont's lark population, for example, by treating only animals with high levels of infestation.
3. Avoid high animal densities as they facilitate the transmission of ectoparasites and reduce stress factors by minimizing the handling and transportation time of the animals.

### Intestinal coccidia

Preventing coccidiosis is based on limiting the ingestion of sporulated oocysts (infective) by larks, so they develop immunity without clinical disease. Therefore, it is also not desirable to completely eliminate exposure to coccidia since releasing non-infected animals would mean they would encounter them in the destination area without prior immunity. However, it is relatively easy for large amounts of oocysts to accumulate in confined spaces if measures are not taken. Translocating healthy animals is also essential to ensure individuals have an adequate immune system. To achieve this, the following measures may be effective:

1. Perform a thorough clinical examination of the animals to determine their health status and only translocate seemingly healthy animals.
2. Administer coccidiostatic medications (single oral dose) to translocated larks before release to prevent high parasite burdens and, therefore, clinical disease (McGill et al., 2010). Ideally, faecal samples should be taken and coprological examinations performed on captured individuals to quantify the amount of coccidia and treat only individuals with high loads. However, this scenario is not possible for the proposed type of translocation (i.e., capture and release occurring on the same day).
3. Avoid high animal densities and ensure maximum hygiene during translocation (capture, handling, and transportation). Disinfection of materials with a 10% sodium hypochlorite solution (bleach) is suitable for destroying environmental oocysts. Reducing stress factors throughout the translocation also plays an important role.

### Hemoparasites

Ideally, the safest way to avoid risks associated with hemoparasites would be to perform diagnostic examinations before transportation and avoid translocating animals infected with potentially pathogenic species. However, this scenario is not possible for the proposed type of translocation (i.e., capture and release occurring on the same day). General measures to minimize the risk of this hazard can be considered:

1. Perform a thorough clinical examination of the animals to determine their health status and only translocate seemingly healthy animals.
2. Minimize stress factors during translocation.

### Aspergillus fumigatus

The most effective way to reduce the risks associated with *A. fumigatus* is 1) to translocate healthy animals, 2) reduce stress during translocation, and 3) reduce the number of environmental spores during transportation. To achieve these objectives, the following options should be considered:

1. Perform a thorough clinical examination of the animals to determine their health status and only translocate seemingly healthy animals.
2. Administer preventive treatments before release (single oral dose of itraconazole). This measure has been used in the translocation of hihi birds (*Notiomystis cincta*), an endangered species in New Zealand (Ewen et al., 2012).
3. Avoid animal accumulation or high animal densities and minimize stress factors such as temperature fluctuations, excessive noise, etc., during transportation, as they can increase the probability of infection.
4. Avoid the use of dirty or mouldy transport boxes and materials during the translocation. It is important to ensure that the transport boxes are clean and dry and have adequate ventilation. In general, ensure the hygiene and disinfection of all materials required for the translocation.
5. There is a recommendation to select release areas with low counts of *Aspergillus spp.* (<100,000 Colony Forming Units per gram of soil) (Ewen et al., 2012). The optimal habitat for the Dupont's lark, including the destination area, is characterized by low humidity levels, which is expected to result in low spore counts.

### Enterobacteria

Ideally, the safest way to avoid the risks associated with enterobacteria would be to conduct diagnostic tests before transportation and avoid translocating animals infected with potentially pathogenic species. However, this scenario is not possible for the type of translocation proposed (i.e., capture and release occurring on the same day). General measures can be considered to minimize the risk of this hazard:

1. Conduct a thorough clinical examination of the animal to determine its health status and only translocate seemingly healthy animals.
2. Minimize stress factors during the translocation and reduce the likelihood of faecal-oral transmission of these bacteria through strict hygiene and biosafety protocols.





3. Treatment of enterobacteria with antibiotics is not recommended as it requires many days of administration, which is challenging in wild animals, and it may favour animals becoming carriers (Daoust & Prescott, 2007).

### Knowledge gaps and research opportunities

While conducting this DRA, a series of knowledge gaps were identified, as shown in Table 3.6. Future research in these areas would enhance the ability to make more informed decisions regarding disease risk.

**Table 2.** Knowledge gaps and measures to reduce uncertainty for each identified hazard.

Hazard	Knowledge gaps	Measures to reduce uncertainty
Ectoparasites	Parasitism level considered normal.	Compare the captured larks to see if some individuals have a higher density of parasites, and classify them into parasitism categories.
Ectoparasites	Presence of new ectoparasites.	Take samples of new parasites (e.g., ticks) to determine the species.
Coccidia	Prevalence of coccidia in the target population.	Captures and recaptures of both translocated individuals and the target population to assess prevalence before and after translocation.
Coccidia	Prevalence of coccidia in the target population.	Captures and recaptures of both translocated individuals and the target population to assess prevalence before and after translocation.
Hemoparasites	Prevalence of hemoparasites in the Dupont's lark, parasite load considered normal, and pathogenic potential.	Blood tests of the captured animals to assess the presence of hemoparasites, the degree of individual infestation, and the presence of symptoms.
Hemoparasites	Vectors present in the area of translocation and their potential as transmitters of hemoparasites.	Use traps to catch and analyse the vectors present in the area, as well as the ectoparasites found in the captured individuals, and detect the presence of parasites in them.
Aspergillosis	Prevalence in the Dupont's lark.	Clinical inspection of the captured animals, and culture of endotracheal washings and radiology in the case of showing symptoms compatible with aspergillosis. Necropsy on all dead animals.

Hazard	Knowledge gaps	Measures to reduce uncertainty
Aspergillosis	Presence of aspergillosis in the environment of the destination area.	Collect air samples by sedimentation, filtration, or impaction in different zones and use kits to measure the concentration of <i>Aspergillus</i> in the medium.
Aspergillosis	Prevalence and abundance in translocated animals and assess disease risk.	Take faecal samples or cloacal swabs from the captured animals and perform the corresponding microbiological studies, genotyping the positive samples.
Enterobacteria	Prevalence in other species in the translocation area.	Necropsy of animals found dead in the area and carry out the corresponding microbiological studies through faecal or sewage samples.

### General recommendations and conclusions

Considering the above, the recommended measures for managing the risks associated with disease in this translocation are shown in Table 3.7. By correctly implementing the proposed measures, the risk of disease associated with this translocation is low. Additionally, the risk of zoonotic disease can be reduced to negligible through the described options. Since uncertainty and knowledge gaps are significant for this translocation, the implementation of a pathogen study and monitoring plan for the population of Dupont’s lark, as well as other passerine species in the destination area, is given a high priority. Evidence from other translocations shows that disease outbreaks may take years to become visible and detectable in wild populations. Therefore, the need to establish a long-term health and population monitoring plan is well reflected.

**Table 3.** Evaluation of different management options and final decision for application in this translocation.

Option	Feasibility	Effectiveness	Explanation	Decision
Pre-release physical exam	High	Moderate	Relatively easy to perform on all individuals to detect animals with signs of disease or with high levels of ectoparasites. These animals should be ruled out for translocation.	Yes
Diagnosis of pre-release pathogens	Low	Low	Pre-release testing is not possible due to the proposed translocation. In addition, most pathogens are excreted intermittently, the tests have low sensitivity, and the significance of a positive result is unknown.	No

ADDITIONAL INFORMATION

Option	Feasibility	Effectiveness	Explanation	Decision
Treatment for pre-release ectoparasites	High	Low	Easy to perform, but it is not 100% effective in a single application, and it is undesirable to eliminate the native ectoparasite community. There are other options for managing this risk. In the event of the detection of disease cases, this measure can be implemented for the next translocated group.	No
Treatment for pre-release coccidia	High	Low	Easy to do, but removing the native coccidia of this species could have undesirable effects on the health of individuals. There are other options for managing this risk. In the event of the detection of disease cases, this measure can be implemented for the next translocated group.	No
Treatment for <i>A. fumigatus</i>	High	Low	Easy to perform, but it can have adverse effects on the health of the animal. There are other options for managing this risk. In the event of the detection of disease cases, this measure can be implemented for the next translocated group.	No
Handling and individual transport of animals	High	High	Easily implemented and is an important method of reducing disease transmission and stress. Each handling and transport bag and box must be exclusive to the same individual.	Yes
Use of new gloves for each individual	High	High	Easy to apply and prevents the transmission of diseases between animals and between animals and people. Gloves (latex or nitrile) should be discarded after contact with one individual and new gloves used for the next individual to be handled.	Yes
Material disinfection	Moderate	High	It can be laborious to implement, but the effectiveness in reducing contamination with faeces and pathogens is high. All material, including bags and boxes used for capture, handling, and transport must be disinfected after use and before being used for another individual. The disinfection of supplementary feeding troughs is an essential part of disinfection because it can be a point of aggregation of individuals and the transmission of pathogens via the faeco-oral route. Applying 10% bleach or VIRKON® for 5-10 minutes is effective in killing most pathogens. It is useful to have extra material to rotate the material in use while the previous one is being disinfected.	Yes
Ensure ventilation of transport bags and boxes	High	High	Easy to apply and very effective in avoiding exposure to <i>A. fumigatus</i> spores.	Yes
Minimize stress during capture, handling, and transport	High	High	Primary method of reducing susceptibility to disease. It can be easily applied with good coordination of the team, avoiding noise and sudden movements, and reducing the time handling and transporting the animals.	Yes

ADDITIONAL INFORMATION

Option	Feasibility	Effectiveness	Explanation	Decision
Avoid trauma and dehydration during capture, handling and transport	High	High	Relatively simple measures can be applied to reduce the risk of some non-infectious hazards. Reduced capture time, effective handling, and the use of transportation methods that do not have racks or iron materials can minimize the risk of trauma. Similarly, the risk of dehydration can be reduced and, in addition, subcutaneous fluids will be administered to all translocated animals after sampling, as described in Section 4.1.5 of this document.	Yes
Pathogen research	Moderate	High	Taking samples during translocation and carrying out diagnostic tests afterwards are essential to increase our knowledge of this species. Although feasibility may be affected by resource limitations, this would increase our ability to make more informed decisions regarding disease risk. Sampling and recommended diagnostic tests are described in Section 4.1.5 of this document.	Yes
Health monitoring	Moderate	High	Although feasibility may be affected by resource constraints, monitoring the health of the Dupont's lark population in the target area is essential. This measure includes monitoring of released animals with emitters. Regular capture and clinical examinations in the destination area for signs of disease may also be considered. Any animal found dead must undergo a complete necropsy and diagnostic tests. Implementing a long-term health monitoring program is crucial to review the DRA, correct management measures, and improve the outcome of subsequent translocations.	Yes

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## APPENDIX 3.4. FEASIBILITY STUDY: ANIMAL WELFARE RISK ANALYSIS

### Introduction

There is growing concern about how conservation activities, including translocations, can affect the welfare of wild animals. This emerging issue is reflected in IUCN translocation guidelines, which recommend incorporating these considerations (IUCN, 2013). Identifying risks to welfare serves a dual purpose. Firstly, maximizing animal welfare during translocation is a responsibility of any human intervention. Secondly, it enhances the success of the translocation from a population perspective as it reduces the likelihood of animals developing disease, as well as improves animal survival and reproduction, and thus the viability of the species (Harrington et al., 2022).

Animal welfare refers to how well an animal adapts to, or copes with, the conditions in which it lives. An animal exhibits good welfare if it is healthy, comfortable, well-nourished, safe, able to express its innate behaviour, and does not suffer unpleasant states such as pain, fear, or distress (World Organization of Animal Health, 2008). In this sense, animal welfare can be evaluated following the “Five Domains” model (see Harvey et al., 2020 for more details). This model allows for identifying compromises in four physical/functional domains (nutrition, environment, health, and behaviour) and in one mental domain that reflects the animal’s affective experiences.

Considering the available information about the species in relation to each domain, a list of potential risks to animal welfare during the current translocation program has been evaluated. These risks have been classified into 1) risks during capture, 2) risks during transport, and 3) risks after release, following the suggestions of Harrington et al. (2022). The original list of risks has been reduced since the current translocation does not include a captivity phase for the animals. Welfare risks related to health (Domain 3) are only briefly mentioned in this section as they have been extensively analysed, and risk mitigation measures are proposed in Section 3.3. It should be noted that animal mortality is not considered a welfare issue per se, but rather the suffering that precedes it.

### Results

#### Risks to welfare during capture

- **Distress, fear, or anxiety due to capture and handling: high probability.**

Justification: The team’s experience indicates that this species displays very docile behaviour in capture bags and during handling. Although this could be interpreted as a lack of stress, many prey species have mechanisms to hide signs of stress or pain to confuse predators. Therefore, the absence or difficulty in recognising signs of stress is not a reliable indicator in this case. Following a precautionary approach, we must assume that capture and handling are highly stressful processes and may cause anxiety and fear in any wild animal.



- Injuries due to capture and handling: **moderate probability.**

Justification: The team's experience indicates that the occurrence of injuries during capture and handling is very low (<1 in 500 individuals; personal data). This species displays very docile behaviour in capture bags and during handling. Therefore, it is not expected that the animals will exhibit signs of struggle that could result in injuries.

- Death due to capture and handling: **low probability.**

Justification: The team's experience indicates that the occurrence of death during capture and handling is very low (2 in 500 individuals, due to predation on individuals in traps; personal data).

- Negative impacts on the source population due to the removal of individuals for translocation: **negligible probability.**

Justification: As it is not a social species, no welfare issues related to the disruption of bonds or social isolation in the source population are expected.

Risk mitigation options: As mentioned earlier, the field team has extensive experience in the capture and handling of this species. Therefore, these procedures will always be carried out by expert professionals, and their duration will be minimized as much as possible. These corrective measures are more extensively detailed in other sections of this document (Section 3.3 and 4.1).

### Risks to welfare during transport

- Distress, fear, or anxiety due to transport: **high probability.**
- Injuries due to transport: **moderate probability.**
- Death due to capture and handling: **low probability.**

Justification: See previous sections.

- Thermal, ventilation, or motion-related discomfort during transport: **high probability.**

Justification: The temperature conditions during transport will differ from those in the source environment. Additionally, ventilation may be compromised within the transport boxes, and the motion of the vehicle can cause discomfort. All these factors may make the transport environment challenging for the individuals.

Risk mitigation options: Transport of the birds will be carried out in individual cardboard boxes with perforations on the sides. This will allow confining the animals without immobilizing them, maximizing ventilation, and minimizing the risk of their overheating (Ewen et al., 2018). Furthermore, the transport phase will last a maximum of 6 hours (including capture, handling, travel, marking, and

release of the animals). The release of excessively stressed or injured animals will be avoided through a second clinical examination at the release site. These corrective measures are more extensively detailed in other sections of this document (Section 3.3 and 4.2).

### Risks to welfare after release

- Distress, fear, or anxiety due to marking and monitoring methods: **high probability.**

Justification: Following a precautionary approach, we assume that the fitting of VHF transmitters is a stressful process that can cause anxiety and fear in any wild animal. Additionally, transmitters could also affect an animal's normal behaviour, such as increasing grooming time or dedicating time to try to remove the device, which may come at the expense of other behaviours. On the other hand, rings are a routine marking method in birds that, when placed by expert personnel, are extremely safe and rarely have effects on behaviour.

- Injuries due to marking and monitoring methods: **moderate probability.**

Justification: The team has never recorded an injury caused by a ring or VHF transmitter. However, there are records of injuries caused by transmitters, both direct and indirect, when individuals self-harm trying to detach the transmitter (Geen et al., 2019). On the other hand, rings are a routine marking method in birds that, when placed by expert personnel, are extremely safe and rarely result in injuries.

- Death due to marking and monitoring methods: **low probability.**

Justification: The team has never recorded a death caused by a ring or VHF transmitter. However, there are records of deaths caused by transmitters in the literature (Geen et al., 2019), making the risk not negligible.

- Distress due to the release of individuals without their social group: **low probability.**

Justification: As it is not a social species, no welfare issues related to breaking social bonds or social isolation are expected in the released animals.

- Distress due to the release of individuals in groups in solitary species: **low probability.**

Justification: While theoretically possible, no welfare effects of this type have been recorded in other translocations (Harrington et al., 2022). The low density of animals in the release area and the rapid dispersal of the released animals are expected to avoid negative effects.

- Distress, injuries, or death due to capture and handling for monitoring: **low probability.**

Justification: Due to the behaviour of this species and the low recapture rates, monitoring will primarily be conducted remotely, using transmitters. If the team considers capture necessary in the release area, the welfare risks would be equivalent to those presented previously in the section “Risks to welfare during capture”.

- Distress, health problems, or death due to lack of post-release support: **moderate probability.**

Justification: The translocation plan does not include support actions after release, and the animals will be released in a “hard release” manner. Previous studies suggest that hard release is the best method for small territorial insectivorous birds (Lovegrove & Veitch, 1994; Lovegrove, 1996). There is uncertainty about the behaviour of the released individuals, specifically whether they will remain in the release area or more frequently tend to disperse or move back to the origin site, which would result in individual loss and partial or total translocation failure. “Soft release” methods, such as an acclimation phase in cages in the release area, could reduce the risk of dispersal (Appendix 3.1). However, this could also cause welfare problems, increasing accumulated stress and potentially causing health or behavioural problems (McEwen, 1998; Dickens et al., 2010). Given the uncertain risk/benefit balance for the animals, soft release methods require additional resources and effort for preparation and maintenance. Therefore, the present plan proposes to initially follow a hard release method, which can be subjected to necessary modifications in subsequent years on the basis of the results of post-monitoring. Initially, the placement of feeders with speakers to improve persistence in the release area and to supplement feeding was considered. However, this option was discarded as the playback of recorded songs could cause stress to local individuals, and the feeders may act as a congregation site for animals and pose a risk of pathogen transmission.

- Distress, health problems, or death due to unknown, unresolved, or inadequately mitigated threats: **moderate probability.**

Justification: The release areas have been selected to maximize habitat suitability. If necessary, the habitat will be restored and prepared before release, based on evidence collected during the preceding LIFE Ricotí project. However, there is still uncertainty regarding the nature and magnitude of threats facing the species and the most effective actions to mitigate them. Therefore, welfare problems for animals in this regard may arise. The present project has an exploratory approach and deliberately seeks to identify these problems.

Options for risk mitigation: regarding risks associated with marking methods, they will be exclusively carried out by experienced professionals (Section 5.2). The transmitters will never exceed 3% of the individual’s body weight, as it has been demonstrated that this greatly reduces the incidence of behavioural and health problems (Geen et al., 2019). All released animals will be monitored, providing information on behaviour, reproduction, and survival. Additionally, the field team will conduct

observations in the release area, allowing for the detection of sick or dead animals. This data will help refine release protocols and species threat mitigation. In the case of a hard release, this may include the incorporation of an acclimation phase and/or supplemental feeding in the release area. In the event of implementing new mitigation measures for threats that could impact animal welfare (e.g., predator control, feral cats), they will be carried out following all legal procedures and best practice protocols, and welfare risks will be re-evaluated. It is important to emphasize that the current translocation program has an emergency or exit plan in case these risks become unacceptable (Section 3.5).

In summary, and as a conclusion, there is a **moderate risk to animal welfare** in the present translocation of the Ricotí lark. The exact magnitude and impacts of these risks are unknown as there have been no prior attempts at translocating this species. Risks to welfare during the capture and transportation phases will be mitigated through the extensive experience of the team in capturing and handling this species, and by following the best practice protocols developed in other translocations. Risks to welfare after release are more difficult to manage as they will remain uncertain until experience is accumulated through the carrying out of translocations. The monitoring protocol described in this document (Section 5) is designed to identify risk factors, and the adaptive approach (Section 3.5) will allow for the correction of welfare issues in balance with the project's objectives.



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