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Methodological and Ideological Options

Rationalizing *ex situ* collection of reproductive materials for endangered livestock breed conservation

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ABSTRACT

Improvements in *ex situ* storage of genetic and reproductive materials offer an alternative for endangered livestock breed conservation, but collections should be optimized cost-effectively to avoid duplication, and with reference to the sustainability of *in situ* breeds. We developed a multi-period chance-constrained optimization model to rationalize collections of endangered livestock breeds at risk of *in situ* extinction in Spain. The model configures collections by determining the least-cost optimal collection locations, timing and material quantities (semen doses). A decision variable defining an "acceptable level of risk" allows decision makers to specify tolerable levels of *in situ* breed endangerment when taking *ex situ* collection and storage decisions. Using data from 18 gene banks we demonstrate how collections can be rationalized, and derive cost curves relating marginal *ex situ* collection cost and accepted probability of *in situ* extinction covering the period 2018 to 2060. The modelling framework can be replicated in countries seeking to rationalize *ex situ* collections under limited conservation budgets and uncertain *in situ* extinction risks.

1. Introduction

Globally, commercial livestock production is dominated by a limited number of specialized breeds, displacing many local breeds, which are *at risk* of extinction (Hopkin 2007; Tisdell 2003). This concentration potentially forecloses future breeding options and the adaptability to changing market, and production environments (Hoffmann 2010). Improvements in *ex situ* storage of germplasm (cryoconserved semen, ova, embryos or any cell with reproductive potential), are a complementary (to *in situ*) approach for conserving genetic diversity between and within breeds and maintaining breed and trait restoration options open to countries (Delgado Bermejo et al. 2019). Due to their economic and socio-cultural significance, commercial livestock breeds are at the forefront of *ex situ* conservation efforts undertaken in many countries and regions in both public and privately funded collections (Leroy et al. 2019). While there has been considerable attention on the technical feasibility of storage and use of materials (Morrell and Mayer 2017), breed prioritization algorithms (Simianer et al. 2003) and to some extent the ethical and governance implications (Farhadinia et al. 2020), there has been less discussion about the need to rationalize collections, using criteria that might be expected to guide efficient investment decisions (Tisdell 2015). This entails clear articulation of the relevant cost-effectiveness criteria, including the configuration of national and international collections to avoid redundancy, and accounting for evolving *in situ* conditions.

Addressing this problem, De Oliveira Silva et al. (2018) explored mathematical optimization as an approach to illustrate how rationalization criteria applied at a European scale might entail fewer or even a single collection. Focussing mainly on variation in collection costs, the analysis did not account for specific national objectives and obvious institutional constraints that might drive *ex situ* priorities according to sub-national *in situ* breed risk status. To develop this idea with a specific national case study, this paper develops a stochastic chance-constrained linear programming (SCLP) model to rationalize collections under projected risks of *in situ* extinction. The model is a novel approach to rationalize collections by determining the optimal collection location, timing and quantity of material (semen doses). Using Spanish breed status data, the model introduces an exogenous decision constraint, an

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'acceptable level of risk', allowing planners to determine national intertemporal *ex situ* investment decisions using information on subnational *in situ* breed status and accounting for uncertainty within *in situ* populations.

This paper is structured as follows. Section 2 considers drivers of endangerment the current status of Spanish livestock breed conservation and stated policy objectives. Section 3 presents methods details the modelling approach to address policy, and outlines the data used in the analysis. Section 4 presents extinction risk scenarios and the cost-analysis results. Finally, Section 5 discusses results and policy implications and Section 6 offers conclusions.

2. Spanish conservation efforts

As in many countries, a combination of market and policy failures has inadvertently driven diversity out of Spanish agricultural production (Perea et al. 2018). Market failure implies that a limited number of genetic traits have typically been favoured by producers responding to market signals, leading to concentrated breeding effort focused on high performing breeds containing these traits (Tisdell 2003). The same signals tend not to reward traits associated with cultural or other non-market values, including the option value related to future contingencies like a more extreme climate. Policy failures can exacerbate this bias, with governments facilitating access to market for producers of a few specialized breeds at the expense of niche producers seeking to maintain the status of rare autochthonous or marginal breeds often for cultural reasons. While there has been some evolution in policy objectives to target the critical status and to support the conservation and use of some breeds, decades of neglect mean that 84% (139 of 156) autochthonous Spanish breeds are currently classified as specially-protected or in danger of extinction (MAPA 2010).

Recognising the pressures and value of endangered local breeds, a Royal Decree (Spain 2008)

established a national action plan to reinforce and coordinate the *in situ* and *ex situ* actions for breed preservation by public institutions (universities, research organizations), breed associations and private companies. This Decree designates national references centres for different elements of livestock conservation (including genetics and reproductive materials) and the maintenance of gene banks (to store germplasm), supporting rare breed conservation located in 9 of the 17 autonomous regions. There is also provision for a National Germplasm Collection (BNG) as a central backup for coverage in regional collections and those held by breed associations (MAPA 2010).

Despite these efforts, more than half of the autochthonous breeds are currently classified as endangered, and approximately half of these are at high risk of extinction (MAPA 2020). Two thirds of endangered breeds are not included in existing gene bank collections (Tejerina 2015). There is also considerable redundancy in terms of overlapping collections, and the national plan acknowledges the need for coordination of autonomous regional facilities covering reproduction and genetic diversity. A significant challenge for the national plan is how to rationalize, coordinate and re configure collection efforts to maximize collected breeds for a given budget constraint, and given the uncertain status of *in situ* resources. This objective can be re-framed as a cost-effectiveness analysis informed by defined objectives that are combined and analysed in a mathematical optimization approach. The following analysis develops a multi-period stochastic chance-constrained linear programming to rationalize collections for projected *in situ* extinction risks. Chance-constrained programming (Charnes and Cooper 1959) is used to handle uncertainty in mathematical models by specifying a confidence level, which is desired for an uncertain constraint to hold; in this case, the uncertainty of *in situ* population projections and thus, risk of extinction.

3. Material and methods

3.1. Rationalizing collections

3.1.1. Collection criteria

The analysis considers collection of doses of frozen semen, the most abundant material in gene banks, a dose being the unit used for a single insemination. Following collection of semen doses, cryopreservation involves dilution in so-called extenders (containing nutrients and protectants), packaging (typically in individually identified plastic straws of in 0.25- or 0.5-mL), cooling and freezing, and subsequent storage in liquid nitrogen, at −196 Celsius (Sieme and Oldenhof 2015).

Semen collection is informed by a global objective to provide a safety margin for breed reconstruction, being a sufficient quantity of semen doses for breed reconstitution, recommended as a 150% breed replacement plan. As sperm concentration varies widely across different species, we use a generic recommendation (FAO 2012) on the volume of semen required per collection for the specified reconstruction target. This is equivalent to 2075 doses of 0.25 mL for cattle, goat and sheep, 775 doses of 8 mL for equine (horse and donkey), 725 doses of 8 mL for pig and 3000 doses of 0.25 mL for chicken collected from 25 donors. For simplification, regardless of species, we convert doses into the equivalent number of straws of 0.25 mL of semen.

In addition to required doses/straws for reconstruction, *ex situ* collection implies an *in situ* donor, in this case and hence endangered breed of evolving population status.

The material collection decision can be determined with reference to the size and proportion of reproductive males and females. FAO guidance (Table 1), indicate the number of breeding females (*NbF*) and breeding males (*NbM*) as a proxy for endangerment level and thus a priority for *ex situ* attention.

We use these to define extinction risk scenarios, *i.e.*, scenario *CTC* for $NbF \leq 100$ and *EDG* for $NbF \leq 1000$ breeding females. As future population size is unknown, we define a function, $Risk_{NbF,t,b} \in [0,1]$,representing the probability that the population of a livestock breed *b* being below the population status *NbF* in year *t*. To avoid redundancy we need only consider *NbF* as the *NbM*/*NbF* ratio will hold for any *NbF* in the dataset we used. Thus, by setting an arbitrary accepted level of risk (r) $\in [0,1]$, we can determine the year in which the endangered breed material should be collected to prevent extinction, the latter being where the number of individuals is below the critical population reproduction threshold.

Numbered steps in Fig. 1 illustrate the modelled *ex situ* collection decision criteria based on *r* and $Risk_{NbF,t,b}$ (1). The decision to collect a breed *b* in year *t* depends on whether the projected population size has an extinction probability lower than *r* (2). If the probability is lower than *r* in year t, then no genetic material (semen) is collected (3). If the extinction probability is greater than *r* in year *t*, the model determines whether breed *b* is already in a gene bank (4). If not, the model determines the cost minimizing collection and storage strategy for a minimum number of doses, where $\mu_b/2$ will be stored as backup material in the national gene bank (5). If an endangered breed is already stored in a gene bank, the model will check whether the current doses meet the minimum amount of μ. If not, doses must be collected so that there is a total of μ doses across the gene banks, with half in the backup (6). The model will produce an efficient cost curves mapping accepted risk levels

Table 1
FAO risk status classification used in modelling scenarios.

Modelled scenario	Breed status	Number of breeding females (*NbF*)	Number of breeding males (*NbM*)
Critical (*CTC*)	Critical	≤100	≤5
Endangered (*EDG*)	Endangered	>100 and ≤ 1000	≤20

*Source: Adapted from FAO (2015).

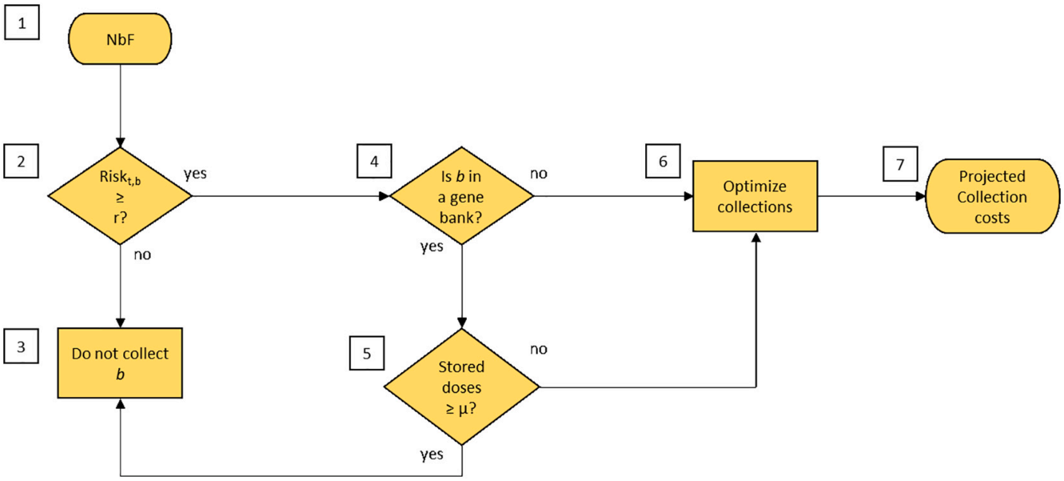

Fig. 1. Material collection decision criteria for endangered breeds.

r against collection/storage costs (7).

3.2. Optimizing collections

The stochastic chance-constrained linear programming (SCLP) seeks to minimize the costs of the collection subject to cryotank capacities, spatial distribution of breeds, distance to the gene banks, and uncertainty around *in situ* projections.

Based on an initial allocation of genetic materials (semen doses in 2018) in the gene banks, the multi-period model determines the number of semen doses to collect, where to collect (in which Spanish autonomous region), where to store (which gene bank location), and when to collect (year) to prevent extinction of breeds. We run the model for 52 years, from 2009 to 2060, with collections starting from 2018 to 2060.

The SCLP model is described in terms of sets and indexes, economic parameters, decision variables, objective function and constraints (Table 2).

$$Min\ cost = min\sum_{(t,gb)})U_{t,gb} \tag{1}$$

Subject to:

$$Y_{t,sb,gb} = \sum_{c} X_{t,sb,gb,c} \tag{2}$$

$$S_{t,b,gb} = A_{b,gb}, t = 1 \tag{3}$$

$$S_{t,b,gb} = S_{t-1,b,gb} + 0.5\sum_{j} Y_{t,sb,j}, gb = 1 \tag{4}$$

$$S_{t,b,gb} = S_{t-1,b,gb} + 0.5\sum_{j} Y_{t,sb,gb}, gb \neq 1 \tag{5}$$

$$V_{t,gb} = \eta\sum_{(sb,c)} \frac{2D_{c(sb),i}}{\gamma} X_{t,sb,gb,c} \tag{6}$$

$$Z_{t,gb} = \lambda\sum_{(sb,c)} X_{t,sb,gb,c} + V_{t,gb} \tag{7}$$

$$U_{t,gb} = m_{gb} + Z_{t,gb} \tag{8}$$

$$X_{t,sb,gb,c} \leq \beta_b G_{gb,t,c} \tag{9}$$

$$\sum_{b} S_{t,b,gb} \leq c_{gb} \tag{10}$$

$$X_{t,sb,gb,c} = 0, gb = 1 \tag{11}$$

$$Pr\left(\sum_{gb} S_{t,sb,gb} \geq \mu_{sb} |\ Risk_{\mathrm{NbF},t,sb} \geq r\right) = 1 \tag{12}$$

The model objective function (1) is equivalent to minimizing the total cost of all gene banks over the planned period. Eq. 2 defines the number of stored doses in a gene bank as a function of doses collected across the different Spanish autonomous regions. Eq. (3) is used to initialize the stored doses in the gene banks. Eqs. (4) and (5) represent the dynamics of semen storage over time. The number of doses in year *t* is the same as year *t-1* plus the number of collected doses, half of which are allocated to the central (backup) gene bank (5). Eq. (6) represents travel costs (excluding collections costs), which are proportional to the total number of collected doses divided by the number collected per trip and the distance (round trip) from the gene banks to the location of donor animals. Eq. (7) represents the total collection costs, given by the semen collection costs, proportional to the number of collected doses, and the travel costs. Eq. (8) is the total annual cost for a gene bank, comprised of fixed costs and total collection costs. Eq. (9) is used to link the number of collected doses to wherever the animals are located, *i.e.*, the number of collected doses cannot be greater than the number of animals in a location multiplied by recommended/minimum doses per animal.

Eq. (10) limits the number of stored doses based on the current capacity of cryotanks. In the case of Spain, much investment has been done already in cryotanks, so that increasing the number of tanks is not an option to be considered. Thus, the storage capacity as well as the associated costs are fixed in our model.

Eq. (11) imposes the backup gene bank requirement ($gb = 1$), which does not itself collect semen doses.

The chance-constraint (CC) Eq. (12), links the stored/collected doses decision variable with *in situ* population projections. The equation guarantees the model will collect the recommended amount of semen doses whenever the probability of extinction is equal or greater than an arbitrary value $r \in [0,1]$.

A linear programming trick (AIMMS 2018) helps to reformulate the SCLP model as a deterministic equivalent LP model. The probabilistic constraint (12) can be replaced by:

$$S_{t,sb,gb} \geq \begin{cases} \mu_b, if\ Risk_{\mathrm{NbF},t,sb} \geq r \\ 0\ if\ Risk_{\mathrm{NbF},t,sb} < r \end{cases} \tag{13}$$

And:

Table 2

Model sets, parameters and decision variables.

Indexes	Description	Value	Unit
B	Breeds	Table S1**	
Sb	Spanish breeds	Table S2**	
gb,j	Gene banks	Table 3	
c,i	Spanish region	Table S2**	
c(gb)	Index function representing which region *c* is gene bank *gb*	Table 3	
T	Time		
Parameters	Description	Value	Unit
T	Storage time	52	Years
c_{gb}	Total capacity of cryotanks in gene bank *gb*	Table 3	straws (0.25 mL)
$A_{b,gb}$	Number of doses of breed *b* currently stored in gene bank *gb*	Table 3	straws (0.25 mL)
m_{gb}	Fixed costs of gene bank *gb* (liquid N and other storing costs)	Table 3	€.year^{-1}
cc_{gb}	Semen collection cost of gene bank *gb*	1.0	€.straw^{-1}
β_b	Recommended number of collected doses per head	Table S10**	doses.head^{-1}
Α	Recommended number of donors	25	head
β_b	Recommended number of doses per breed collection	α *μb	straws
γ	Average number of collected doses per travel	β_b	straws
$D_{c,i}$	Distance between centre of region *c* and centre of region *i*	Table S3**	km
η	Average fuel costs	1	€.km^{-1}
$G_{sb,t,c}$	Population size of breeding males of breed *sb* in year *t* in city *c*	Table S4**	head
$NbF_{sb,t}$	Population size of breeding females of breed *sb* in year *t*	Table S5**	head
$L_{sb,t}$	Lower bound of $NbF_{sb,t}$	Table S6**	head
$U_{sb,t}$	Upper bound of $NbF_{sb,t}$	Table S7**	head
$Prob_{N,t,b}$	Probability of the population size of breed *b* being below the population status CTC or EDG in year *t*	Table S8-S9**	Dimensionless
Variables	Description	Value	Unit
$X_{t,sb,gb,c}$	Number of semen doses collected of breed sb in year t by gene bank *gb* in city $_c$		straws
Yt,sb,gb	Number of semen doses collected of breed *sb* in year *t* by gene bank *gb*		straws
$S_{t,b,gb}$	Number of stored semen doses of breed *b* in year *t* in gene bank *gb*		straws
$V_{t,gb}$	Travel costs of gene bank *gb* in year t		€
$Z_{t,gb}$	Collection costs of gene bank *gb* in year t		€
$U_{t,gb}$	Total annual costs of gene banks *gb* in year t		€

** Supplementary tables are published as a separate Data in Brief article, see De Oliveira Silva et al. (2020).

$$Risk_{t,sb}\begin{cases}1, \text{if NbF} - U_{t,sb} > 0\\ 0, if\ \text{NbF} - L_{t,sb} > 0\\ \dfrac{\text{NbF} - L_{t,sb}}{U_{t,sb} - L_{t,sb}}, otherwise\end{cases} \tag{14}$$

We use scenarios CTC and *EDG* (Table 1) to decide when to intervene for conservation of natural populations; $U_{t,sb}$ and $L_{t,sb}$ are the upper and lower confidence bounds (with 95% confidence) of a linear regression using Census data from 2009 to 2018 (MAPA 2020). Fig. 2 illustrates the technique for transforming (12), with an uncertainty bound in the right-hand side into a purely deterministic constraint.

3.3. Data

Census data were provided by the National Information System of Spanish Livestock Breeds (ARCA) held by the Ministry of Agriculture, Fisheries and Food (MAPA 2020) containing times series for *NbF* and *NbM* of all autochthonous breeds from 2009 to 2018. The data and the geographic distribution of the animals is uploaded to the ARCA web portal annually by the breed associations (See Table S2 in De Oliveira Silva et al. (2020)). The data cover all Spanish autochthonous breeds for seven livestock species (the number of breeds classified as non-endangered/endangered for each species in parentheses), cattle (8/31), sheep (36/8), goats (20/3), pigs (3/12), chicken (1/16), equine (horse and donkey) (1/14).

A survey was also distributed to 8 autonomous community gene banks and another 10 gene banks selected by geographical location and/or biological material stored. The survey gathered information on species germplasm and breeds and cost data, including fixed maintenance cost of cryotanks and for upgrading their capacity, labour costs, materials and equipment. Information is summarised in Table 3.

To plan *ex situ* collections from 2018 to 2060 we used data for 210 livestock breeds, of which 179 are Spanish autochthonous breeds, 18 gene banks based in 15 different locations. Each bank can collect samples from 50 *in situ* locations (Spanish provinces). We used census data from 2009 to 2018 for the linear regression (Parameters $G_{t,sb,c}$ and $Risk_{t,sb}$) and the production bounds calculated using Matlab (Radi and El Hami 2018). Gene bank storage dynamics are simulated from 2009, while collections start from the reference year of 2018 (most recent data). The deterministic version of the SCLP model consisted of 7.62 M variables per 7.62 M constraints and was implemented in AIMMS algebraic language (Bisschop 2018) and solved by CPLEX12.8 (IBM Corp. and IBM 2009).

Cost curves of least-cost collections mapped the values of the least cost objective function (Eq. 1) against *r*, varying from 0 to 1.

4. Results

The results involve three parts; uncertainty analysis of the *in situ* data and projections, least cost collection strategies and allocation of genetic materials in the gene banks, and sensitivity analysis of "acceptable level of risk" *versus* total collection costs.

4.1. In situ projections

Currently (2018), 10/179 (≈ 6%) of all Spanish livestock breeds are at *CTC* risk, while 62/179 (35%) are at *EDG* risk. As 2018 is based on observed data, we assume this is known with 100% probability. However, it is uncertain how many breeds will be at *CTC* or *EDG* in the next 20–30 years.

For future *in situ* populations (2018 to 2060), we derive probabilistic projections for future population status, *i.e.*, the number of breeds at *CTC* (Fig. 3a & b) or *EDG* (Fig. 3a & b) as a function of probabilities of the breeds being at those scenarios (See Appendix for detailed projections). The probabilistic projections are based on census data from 2009 to 2018 of *NbF* for 179 breeds including cattle (45 breeds), sheep (52), goat (22), equine (horse + donkey) (25), pig (18) and chicken (17).

We describe the projected probability curves by taking the reference years of 2020 and 2040 to capture the simplified evolving status of the livestock breeds in terms of recovery and/or increased endangerment levels.

Figs. 3a & b show that *CTC* breeds are unlikely to improve the endangerment status as the number of breeds in 2040 are higher than in 2020, regardless the risk scenario (5% to 95% probability).

There are 13 livestock breeds at a 50% probability of being at *CTC* in 2020 (Fig. 3a), and the number is expected to increase to 24 by 2040 (Fig. 3b), *i.e.*, an increase of nearly 100% of *CTC* breeds. Similarly, we estimate there is a 75% probability that 13/179 (7%) breeds will be at

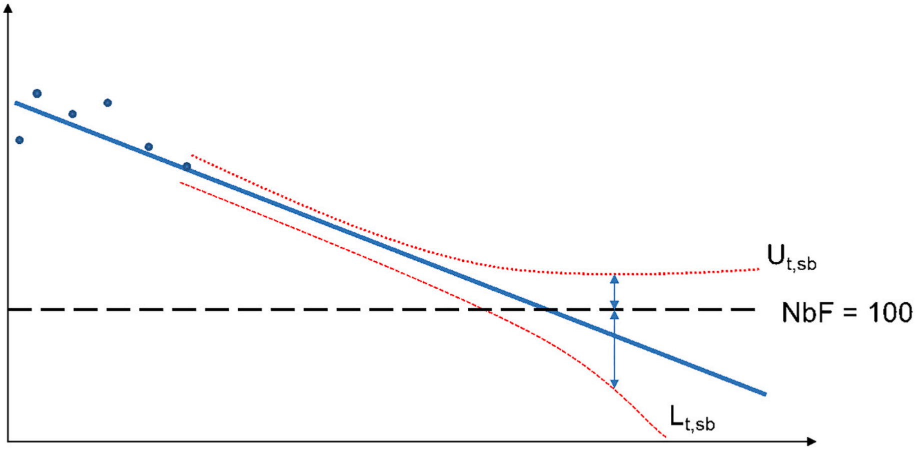

Fig. 2. Calculation of extinction risks based on population status (N) and regression uncertainty bounds.

Table 3
Gene bank cost and capacity data.

ID	City	Capacity (straws[1] of 0.25 mL)	Current use (straws of 0.25 mL)	% Capacity used (%)[1]	(EUR/ yr)
GB1	Madrid	550,000	72,618	13%	13,000
GB2	Madrid	48,000	31,308	65%	4200
GB3	Ciudad_Real	65,000	42,723	66%	4830.53
GB4	Zaragoza	780,000	633,821	81%	25,000
GB5	Islas Baleares	16,000	15,074	94%	6000
GB6	Badajoz	1,500,000	1,071,951	71%	100,000
GB7	Leon	1,600,000	853,097	53%	30,000
GB8	Asturias	2,740,000	395,982	14%	20,000
GB9	Ourense	780,000	730,425	94%	50,000
GB10	Valencia	4899	4605	94%	314
GB11	Murcia	532	500	94%	34
GB12	Barcelona	25,347	23,826	94%	1622
GB13	Toledo	87,234	82,000	94%	5583
GB14	Cordoba	9324	8765	94%	597
GB15	Bilbao	2,826,276	2,656,699	94%	180,882
GB16	Madrid	277,232	260,598	94%	17,743
GB17	Valladolid	5428	5102	94%	347
GB18	Ciudad Real	55,741	52,397	94%	3567

[1] To fill data gaps for GB10–18 (on capacity and fixed costs), we assume "worst case" of 94% usage and that fixed costs are proportional to the capacity, where costs/capacity = EUR 64/straw is calculated as the median of G1 to G9.

CTC by 2040.

This is particularly concerning for sheep, with only 2 breeds at *CTC* in 2020 but expected to increase to 10 (with a 50% probability) by 2040 (Fig. 3a & b). Figs. 3a & b also shows that while pig, chicken and sheep are the most critical species, in descending order in 2020, by 2040, sheep, cattle and goat are the species requiring most *in situ* and *ex situ* attention.

In comparison to *CTC*, Figs. 3c & d show that breeds at the lesser r *EDG* risk are likely to partially recover over the same period, *i.e.*, we project an overall smaller number of breeds at *EDG* by 2040 than in 2020. While we project 54 breeds with a 75% probability of being at *EDG* in 2020, that figure reduces to 34 breeds by 2040. This is particularly true for breeds with higher probability of being at *EDG*; there are 49 breeds with 95% probability of *EDG* in 2020 and only 25 in 2040. Thus, we suggest *ex situ* effort is required for both *CTC* and *EDG* breeds, but the former are at greater risk not just by definition, but by virtue of being on an overall decreasing trend in population size. Furthermore, extinction risks might be higher/lower than our estimates if existing *in situ* programs or climate and environmental conditions are less/more favourable than what has been observed over the period 2009–2018.

4.2. Optimal *ex situ* collections and gene banks usage

Current (2018) breed allocation across 18 Spanish gene banks (GBs) shows that the majority of the stored samples of 200 livestock breeds are from cattle and sheep. The exceptions are GB2 (chicken), GB3 (sheep and horses), GB10 (chicken and horses), GB11 (chicken only) and GB 17 (pigs only) (Fig. 4a). Cattle breeds represent half of the collected livestock breeds (Fig. 4b), and more than 95% of the currently stored samples (in straws of 0.25 mL), followed by sheep breeds (3%) (Fig. 4b). This relative over-representation might be explained by the fact that European cattle breeds have a longer history of insemination activities, and are economically more important.

Although GBs follow the same local breed conservation guidelines there is considerable variability in collection efforts, evidenced by unbalanced collections across the GBs in term of species, breeds and number of straws (Fig. 4a & b).

The central backup GB1 has the highest number of breeds stored (48) but approximately 2% of the total doses (in straws of 0.25 mL). This is an apparent unbalanced ratio of breeds *vs* doses that could compromise the main goal of GB1, *i.e.*, to serve as a national backup. However, there are another implicit variables, *i.e.*, the number of donors per collected breed and the number of doses per donor that explain this apparent distortion of GB1. Other GBs have an excessive number of doses per donor, while GB1 presents a more balanced number of doses per breed, *i.e.*, an average of 2488 cattle doses per breed, collected from an average of 21 donors. These data are close to the recommended number of doses for cattle, *i.e.*, 2075 doses from 25 donors (120 doses per animal) (FAO 2012).

Each of the probabilistic risk scenarios ($r = 5\%$ to 95%) will generate a different collection strategy and costs under *CTC* or *EDG*. For illustrative purposes, we arbitrarily choose a sensible scenario of acceptable level of risk $r = 50\%$ under *CTC* to described how effective (least coast) collections can be planned by using the existing GBs (Fig. 4c-d). Figs. 5c-d compare information on the current and projected efficient *ex situ* collections across the gene banks in terms of number of breeds and doses.

Fig. 4c shows increased cryotank use (2018–2040) for most of the GBs, especially GB3, GB5, GB11 and GB14, including a 50% increase on GB1 backup doses. But in terms of total doses collected per GB, efficient future collections would be concentrated in GB2, GB4, GB6–7 and GB15–16, or the equivalent to around 80% of total number of collected doses (Fig. 4d). 4d also shows that some GBs are barely used for new collections (GB4, GB9–10, and GB16–17), while others collect a single species, *e.g.*, GB9 would collect chicken breeds only, while GB14 collects six of the seven species considered in this study.

Fig. 5 shows the locations of the least-cost collections (2018–2040)

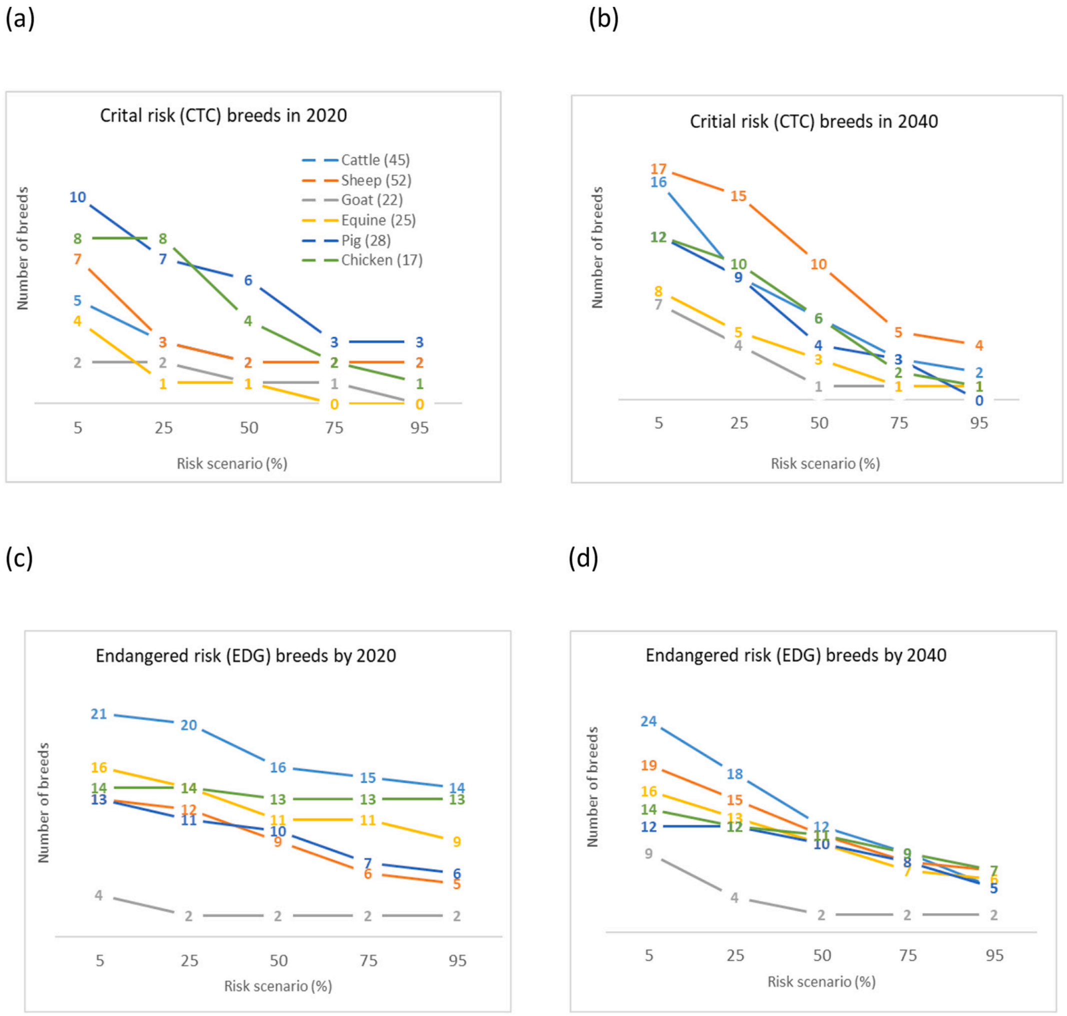

Fig. 3. Projected number of breeds at critical risk (*CTC*) in (a) 2020 and (b) 2040; and endangered breeds (EDG) in (c) 2020 and (d) 2040 as a function of the risk probability scenarios, from 5% to 95%.

under the *CTC* scenario and $r = 50\%$ in number of breeds (a) and number of doses (b).

Fig. 5a shows that breeds that will require urgent conservation intervention can be collected from 24 of 50 provinces in Spain, with the majority of critical breeds being horse and pig concentrated in Madrid (72), followed by Lugo (28), Cantabria (28), Pontevedra (15), Ourence (14), Alava (14) and Barcelona (13), accounting for around 70% of the collections, or 171 of 230. Fig. 5b shows a different regionalised collection and cost configuration when expressed in terms of the number of doses. Despite a high concentration of *CTC* breeds in Madrid (Fig. 5a), these require only 6519 doses (straws of 0.25 mL) compared to 12,309 doses in Badajoz. This is explained by the fact Badajoz collections are mostly full collections of sheep and pig, while Madrid collections are mostly for complementing existing doses across the GBs so that there a minimum of 2075 doses per breed. In contrast, pig collections require a higher number of 5800 doses (in straws of 0.25 ml).

4.3. *Cost analysis of future ex situ collections*

Predicted collection cost is a function of acceptable extinction risks (r) under *CTC* and *EDG* scenarios (Fig. 6). For accepted risk level $r = 5\%$ to 95%, total collection costs covering all species vary from EUR 200, 000 to EUR 54,000, and EUR 274,000 to EUR 195, 000 over the period 2018–2060, for *CTC* and *EDG* respectively. Note that these costs are a fraction of GB operating costs; *i.e.*, maintenance/fixed costs (see Table 3) that totalling EUR 463700 /yr.

Figs. 6a & b illustrate the sensitivity analysis of projected collection costs (2018–2060) as a function of acceptable extinction risks (r) under *CTC* and *EDG* scenarios.

For pigs, higher costs are explained by a relatively large proportion of breeds projected to be at critical status (Fig. 3a), and the relatively high number of recommended straws per collection, *i.e.*, 5800 straws of 0.25 mL compared to 2075 for goats, sheep and cattle. Costs for cattle are fixed, irrespective of r. This is because all but one breed (*Bruna de los Pirineos*), projected to be at critical risk over the next three decades, are already in at least one of the GBs, and costs are thus related to this single breed plus collecting complementary doses so that the GBs contain at least 2075 doses per endangered breed. For equines, costs are sensitive to r up to a plateau, $r = 45\%$ and $r = 20\%$, respectively for horse and donkey breeds.

Collection costs of the seven livestock species are less sensitive to the acceptable level of risk (r) under *EDG* scenario (Fig. 6b). As in *CTC*, costs for cattle and donkeys are fixed, whereas costs for chicken and horses plateau after a threshold value, for the same reasons as in Fig. 6a for donkey and horse breeds.

Under the *EDG* scenario, and accepting an extinction risk level of 25% ($r = 0.25$) for cattle, chicken, equines and sheep, *i.e.*, collecting only the breeds that will be at a probability of being endangered, will slightly reduce the costs for any r bigger than 0.25, from EUR 110, 000 to 100, 000 over the 2018–2060 timescale.

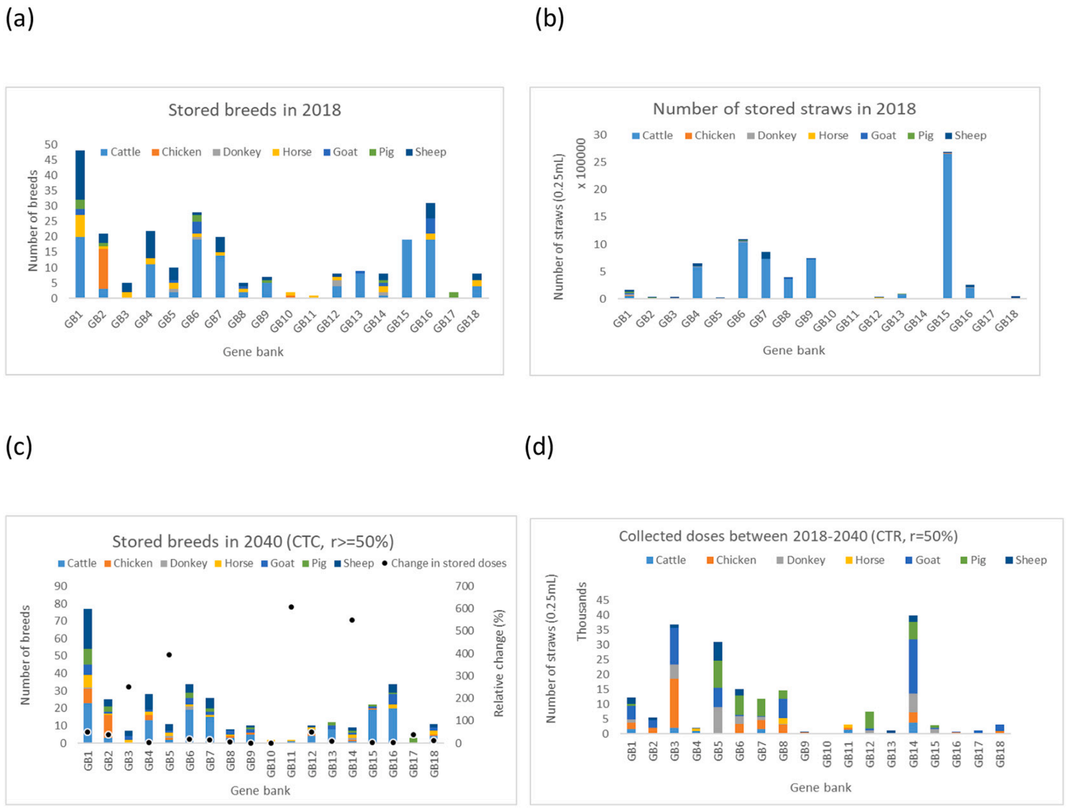

Fig. 4. Breed allocation across the gene banks: (a) current number of breeds and (b) number of semen doses; and (c) projected stored breeds and (d) number of collected doses from 2018 to 2040 under CTC and $r = 50\%$.

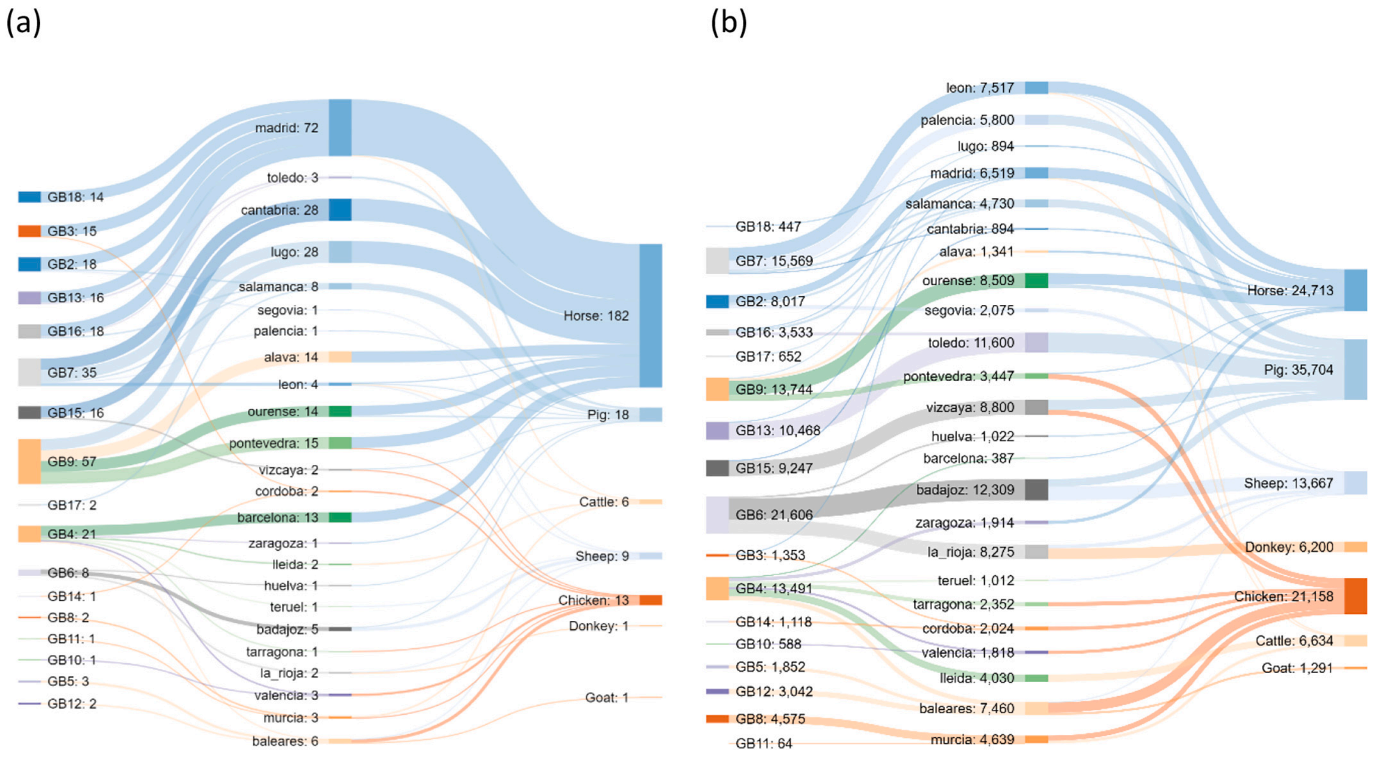

Fig. 5. Allocation and locations of collections in number of breeds (a) and number of semen doses (b) considering $r = 0.5$ and *CTC* scenario over 2018–2040.

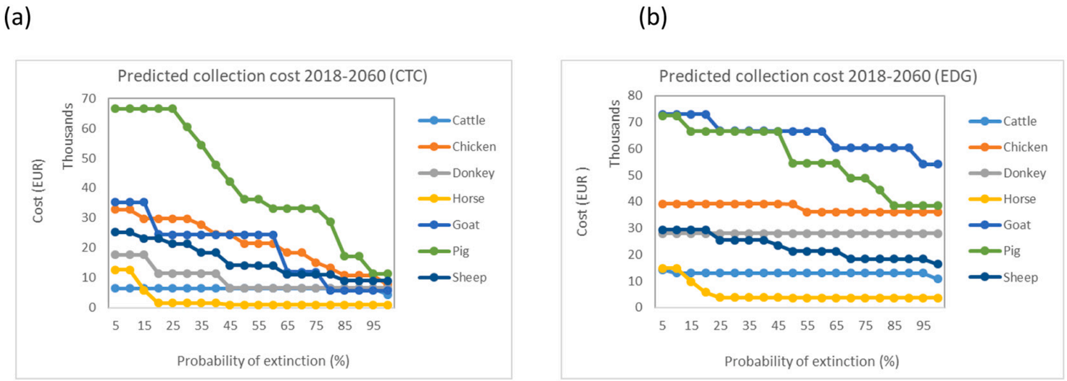

Fig. 6. Cost efficient curves for *ex situ* collections (2018 to 2060) for CTC (a) and EDG (b). The y-axis represents the annual collection costs (excluding fixed costs), the x-axis is the accepted extinction risk before collecting endangered breeds (*r*).

Sensitivity of *CTC* is higher than for *EDG*. Furthermore, the results also suggest the sensitivity between *CTC* and *EDG* scenarios is higher for greater values of *r*. For example, the cost of preserving all breeds for $r =$ 25% under *EDG* is 50% higher than for the same *r* value in the *CTC* scenario (EUR 246 k against EUR 164 k). But the difference increases to 103% and 140%, respectively for $r = 50\%$ and $r = 75\%$. Thus, for lower values of *r* intensifying *ex situ* efforts for *EDG* would be attractive instead of considering *ex situ* investments for breeds that have reached *CTC* status. This affords information to permit a greater number of conserved breeds and lower extinction risks, as all *CTC* breeds are also *EDG*.

5. Discussion

The uncertainly analysis suggests worrying extinction risks for both *CTC* and *EDG* scenarios. However, it suggests that cost-effective planning of future collections means extinction risks can be minimized by using existing local gene banks in coordinated collection efforts, including a national backup collection. The analysis shows how costs are sensitive to *in situ* projections and the “acceptable level of risk”, a decision constraint we introduce to inform future *ex situ* collections investments.

Some further uncertainty arises also from gaps in cost data. The analysis is based predominantly on information from public collections, and some gene banks could not offer complete cost data as well as information related to gene bank capacities (number and volume of cryotanks) and full operating costs. This finding allows us to suggest that there is a need for a consistent templates to facilitate cost comparisons across sites, and a need for industry collaboration for access to data from private collections. Furthermore, collection costs per straw (0.25 mL) were assumed to be the same across gene banks. But this may in fact depend on the species and the availability of skills and equipment for a given species in a given region.

The model parameters have been defined for semen, whereas progress in reproductive biotechnologies make it possible to store other types of material, which may deliver more effective breed recovery, such as pig embryos, gonadal tissues, or primordial germ cells in chickens (Woodcock et al. 2019). Targeting these other materials will change collection costs depending on requisite skills and equipment requirements.

Future work could also model *in situ* decision variables endogenously to explore the trade-off between the complementary roles of *ex* and *in situ* decisions. The modelling could also consider different collection and cryoconservation technologies and reproductive success rates as stochastic variables. Other modelling options possibly accommodated in this framework include breed genetic information, genetic variability and kinship within and across breeds, genetic gain scenarios, different *ex situ* extraction and storage technologies and *in situ* strategies as endogenous variables of the model. For instance, capitalising on data from whole genome sequencing allows us to consider within and across breed genetic diversity, a more precise metric of genetic diversity conservation goals than the numbers of breeds only.

A broader reflection from this work is in terms of the sustainability implications of improved cryoconservation technologies, which increase the scope of human agency in conservation and resource preservation choices. Decisions about what to collect and when, plus a trajectory of falling collection costs arguably blur the weak and strong sustainability boundaries in the context of species conservation.

6. Conclusion

The evolution of cryoconservation methods is increasing the options for *ex situ* conservation as a complement to *in situ* efforts. The overall status of endangered livestock breeds can be enhanced by rationalization to avoid redundancy in *ex situ* resources, and systematic assessment of acceptable *in situ* extinction risks.

This country case study is an example of a modelling framework that can be replicated in other situations and regions with different breeding and conservation objectives. The example illustrates the importance of consistent data collection on conservation costs. In terms of investment appraisal, it offers a tractable solution relative to a cost-benefit analysis and associated challenges of valuing current and future conservation benefits.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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