Drivers of survival in a small mammal of conservation concern: an assessment using extensive genetic non-invasive sampling in fragmented farmland

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Abstract

Although important to guide conservation management, detailed demographic studies on rare or elusive species inhabiting fragmented, human-dominated landscapes are often hampered by the species' low densities, and the logistic and ethical constraints in obtaining reliable information covering large areas. Genetic non-invasive sampling (gNIS) provides cost-effective access to demographic information, though its application to small mammals is still scarce. We used gNIS to infer on the demography of an endemic small mammal, the Cabrera vole (*Microtus cabrerae*), occurring as a spatially-structured population in a 462-ha Mediterranean farmland landscape. We intensively sampled fresh vole feces in four seasons, extracted the DNA, and performed individual identification based on genotypes built using nine microsatellites. We then estimated population size and individual survival relative to environmental variables, controlling for heterogeneity in capture probabilities using capture-mark-recapture modelling. Population size increased during the wet season and decreased during the dry season, while survival remained constant across the study period. Individuals captured along road-verges and around water-bodies survived longer than those captured near agricultural fields. The use of gNIS on a heterogeneous landscape such as our study area allowed us to demonstrate that human land-use activities affect Cabrera vole demographic parameters in Mediterranean farmland, with implications for conservation planning towards its long-term persistence. Our approach can be widely applied to other elusive small mammals of conservation concern, but for which informative demographic data are still scarce.

Keywords

Agricultural intensification; *Capwire*; Cormark-Jolly-Seber; Mediterranean farmland; *Microtus cabrerae*; Road effects.

1. Introduction

Estimating large-scale demographic patterns (e.g. abundance, population growth, survival) of animal species in relation to both individual traits (e.g. sex, age, weight) and environmental factors (e.g. climate or land-use change) is a difficult but necessary goal to understand species ecology and sustain conservation policies (Smallwood and Schonewald, 1998; Williams et al., 2002). This is particularly true for species occurring in agricultural landscapes where major declines in biodiversity due to agricultural intensification have been reported worldwide (Tscharntke et al., 2012). However, achieving these goals is often difficult due to a number of technical, ethical, and logistic constraints in data collection, particularly for species that are rare, elusive, or otherwise hard to capture or observe.

Capture-Mark-Recapture (CMR) is one of the most popular methods to assess demographic parameters in animal populations (Lebreton et al., 1992), and hence to understand species' biology and ecology in different environments (Smallwood and Schonewald, 1998). Traditional CMR studies have been mostly based on live-trapping techniques, which are usually logistically difficult to implement over large spatial and temporal scales and often expensive (Cheng et al., 2017). In addition, because live-trapping implies both physical confinement and handling of animals, it often involves behavioural and physiological responses due to trapping-induced stress (Beja-Pereira et al., 2009; De Bondi et al., 2010). Stress responses can be reduced with the use of minimally invasive techniques such as camera-trapping, which is expected to be more time-efficient than live-trapping and does not require physical capturing and handling of animals (De Bondi et al., 2010; Mondol et al., 2009). However, camera trapping is unsuitable for CMR studies in species that are difficult to morphologically identify at the individual level, which is the case of most small mammal species (Glen et al., 2013). Furthermore, in the case for rare and elusive species, both live- and camera-trapping often yield insufficient data to be used in CMR models, thus hampering proper evaluation of their population status and trends (Burgar et al., 2018; Mondol et al., 2009).

Genetic non-invasive sampling (gNIS) has been increasingly used to estimate demographic parameters of species that are difficult to trap, mainly due to decreased field sampling effort, ever decreasing lab costs, and increasing DNA amplification success (Beja-Pereira et al., 2009; Marucco et al., 2011). Despite its limitations in retrieving information on relevant individual traits like age, body mass, or reproductive condition, gNIS can provide a more cost-effective

92 solution than traditional live-trapping (Cheng et al., 2017; Ferreira et al., 2018). DNA extracted
93 from non-invasive samples (e.g. feces, hairs, feathers) allows the identification of individuals,
94 providing data that can be easily combined with CMR methods to obtain population parameters
95 that otherwise would be difficult to obtain over large spatial scales (Cheng et al., 2017; Petit and
96 Valiere, 2006). However, to date, applications of gNIS in CMR studies have mostly focused on
97 large and medium-sized mammal species, and often provide snapshots of population size
98 estimates rather than variations over time (but see Brøseth et al., 2010 for an example).
99 Furthermore, very few studies have used gNIS to estimate other important population
100 parameters such as survival (Lampa et al., 2015; Marucco et al., 2012; Zielinski et al., 2013). In
101 the case of small mammals, while some recent studies have used gNIS to estimate population
102 density (DeMay et al., 2017; Gillet, 2016; Sabino-Marques et al., 2018) or to infer dispersal
103 (Ferreira et al., 2018; Gillet, 2016), to our knowledge no study has yet explored the application
104 of this method to understand how demographic parameters relate to large-scale environmental
105 variation.

106 In this study, we combined gNIS and CMR methods to assess the seasonal variations in
107 abundance, and to evaluate factors affecting survival probability of an elusive small mammal
108 species in a Mediterranean farmland landscape. We focused on the 'near-threatened', Iberian
109 endemic Cabrera vole (*Microtus cabrerae*, Thomas 1906), for which genotyping protocols based
110 on faecal samples have been recently optimized (Barbosa et al., 2013; Ferreira et al., 2018).
111 Additionally, previous studies have also shown the ability of gNIS to provide reliable density
112 estimates for this species (Sabino-Marques et al., 2018). Based on repeated surveys of Cabrera
113 vole feces, we explored the potential of gNIS to (i) assess the seasonal variation in population
114 abundance; and (ii) estimate capture and survival probabilities in relation to variables reflecting
115 survey conditions (genotyping success and season), individual traits (sex), and local and
116 landscape environmental features. We considered variables that might affect survival both
117 positively (e.g. patch area and presence of water) and negatively (e.g. isolation, patch
118 persistence, interactions with the competitor *Arvicola sapidus*, and human disturbances) (Pita et
119 al., 2014) (see Table 1 for a full description and rationale of covariates considered). Overall, our
120 study illustrates the use of gNIS within a CMR framework, demonstrating its application to
121 retrieve demographic data from elusive small mammals, thus enhancing conservation planning
122 in areas that have been highly modified by human activities.
123

2. Material and Methods

2.1. Study area and species

The study was carried out in a 461.8 ha area within the coastal plateau of south-western Europe, Portugal (37° 21' - 38° 04' N, 08° 51' - 08° 30' W) (Fig. 1). The region is included in the thermo-Mediterranean bioclimatic zone (Rivas-Martínez, 1981), with a mean annual temperature of 16.5°C (monthly temperatures ranging from 6 to 29°C), and an annual rainfall of about 650 mm (of which >80% falls between October and March) (Pita et al., 2007; 2006). The landscape is mostly flat (56-76m above sea level) and land cover is dominated by pastures and annual irrigated crops (Pita et al., 2007). Forest cover is limited to a few woodlots and hedges with pines and eucalyptus, while natural woodlands, shrubs, and marshy vegetation are most frequent along road verges (mostly dirt-roads with low traffic), and in the surroundings of extensive agricultural fields (Pita et al., 2009). Over the past three decades, agricultural practices have been strongly intensified, particularly through the expansion of cultivated land, associated with the frequent use of pesticides and chemical fertilizers, with detrimental impacts on biodiversity (Ferreira and Beja, 2013; Peralta et al., 2016; Pita et al., 2009).

Previous studies in the region have shown that Cabrera voles typically show a metapopulation-like spatial structure (Pita et al., 2007), occurring within damp habitat patches densely covered by tall wet herbs and shrubs along small streams, temporary ponds, field margins, and roadside verges (Pita et al., 2014; 2006). Population densities within patches are typically low (Pita et al., 2011; 2010), and individuals are rarely present in the same area for more than 4 months (Fernández-Salvador et al., 2005b). Within habitat-patches, the Cabrera vole is often organized in monogamous breeding pairs and tend to exhibit home-ranges of only a few hundred square meters (Pita et al., 2010).

2.2. Vole surveys and environmental variables

Following the studies by Ferreira et al. (2018) and Sabino-Marques et al. (2018) conducted previously in a smaller area (78ha) within the same study region, we surveyed Cabrera voles on four main occasions (seasons) along one year: early wet season (EWS, November-December 2013); late wet season (LWS, February-March 2014); early dry season (EDS, May-June 2014); and late dry season (LDS, September-October 2014). In each season, we used a two-step procedure, first identifying and mapping all habitat patches potentially used by Cabrera voles

(damp areas with vegetation patches dominated by dense and tall herbs) using both Bing Maps™ aerial photographs (retrieved in 2012) and ground validation (Fig. 1). Then, a survey was conducted over the whole area of each patch, to detect the presence of the species from its characteristic signs (faecal pellets and grass clippings), and to collect fresh feces for genetic analysis. Within each patch, two samples were collected (if available) every 5 m (in order to maximize the chances of detecting different individuals) (Fig. 1), using sterilized tweezers, and stored in the field in individual 2 mL microtubes with 96% alcohol, and later kept at -20°C until DNA extraction. To minimize cross-contamination from conspecifics, feces were collected from small latrines (<20 faecal pellets). The presence of the competitively superior southern water vole (*Arvicola sapidus*) was recorded based on the presence of similar but larger faecal pellets than those from the Cabrera voles, which are easily recognizable (Peralta et al., 2016). The surveying of the study area followed the same direction (from NW to SE) in each sampling season, such that habitat-patches were sampled following approximately the same order and time-intervals.

All information regarding the voles' habitat, matrix land-uses, and sample geographic location was stored in a vector-based Geographic Information System (GIS; QGIS, version 2.14.10 - Essen, QGIS Development Team, 2016).

2.3. DNA extraction and genotyping

Due to budget restrictions, only a limited number of faecal samples could be analysed. The selection of samples for genetic analyses followed a stepwise approach to reduce costs while aiming to achieve a comprehensive spatial coverage of each patch, hence maximising the number of captures and recaptures of individuals (Ferreira et al., 2018)**. I**n patches where less than six samples were collected, all samples were analysed. In each of the remaining patches, we selected at least 60% of the samples, evenly spread throughout the patch. When more than one sample was collected every 5 m, only one was initially analysed. If genotyping failed for the first sample, the second sample was analysed in order to obtain a minimum number of genotypes per patch of at least 40% of all sampling sites.

Vole DNA was extracted using the E.Z.N.A.® Tissue DNA Kit (OMEGA bio-tek) following the manufacturers' instructions, with an initial digestion step using a lysis washing buffer (Maudet et al., 2004) for 15 minutes at 56°C. Samples were genotyped for a set of nine microsatellites characterized by high levels of polymorphism (Ho = 0.79; He = 0.81), low probability of identity

of unrelated (PI= $3.2x10^{-12}$) and related individuals (PIsibs= $9.2x10^{-5}$), and high probability of exclusion (PE=0.99), and two small sized sex-linked introns (Table A1, Appendix A). These markers have been optimised for application to gNIS of Cabrera voles feces (Ferreira et al., 2018), and provide accurate individual identifications and population estimates (Sabino-Marques et al., 2018). We followed the protocol described by Ferreira et al. (2018), which includes an initial screening of DNA quality using three species-specific microsatellite loci. The samples that amplified for the three loci were then amplified for the additional six microsatellite loci and two sex-linked introns. To account for genotyping errors (e.g. allele dropout and false alleles) and obtain a consensus genotype, each multiplex reaction was replicated a minimum of four times (three times for the sex-linked introns). To confirm species identification, a small fragment of cytochrome-*b* gene was amplified in all genotyped samples following Barbosa et al. (2013). To evaluate eventual biases in the estimation of genotyping success rate due to misidentification of feces in the field, we also performed genetic species identification in at least 20% of the samples that failed during genotyping. The extractions and PCR reactions were conducted in a physically isolated room, where all the equipment was sterilized with bleach and ethanol, and exposed to UV light before and after usage. Negative controls were included in each manipulation, maintaining conditions to monitor and reduce the risk of DNA contamination (Barbosa et al., 2013; Beja-Pereira et al., 2009; Costa et al., 2017). All products were sequenced on a ABI3130 Capillary Sequencer (Applied Biosystems). Allele calling of the microsatellite loci and sex chromosome introns was performed using GeneMapper (v.4.0; Applied Biosystems). Cytochrome-*b* gene sequences were analysed in Geneious 8 (Kearse et al., 2012).

Consensus genotypes for the successfully genotyped samples were obtained by analysing all replicate genotypes with Gimlet v.1.3.3 (Valiere, 2002). For genotypes differing only by one or two loci or with up to two missing data, additional PCR replicates were performed to complete genotypes with missing data, and to check for genotyping errors. Consensus genotypes for each sample were then compared with each other to identify individuals. Following the criteria detailed in Ferreira et al. (2018) and Sabino-Marques et al. (2018), only samples that differed in more than two loci were assigned as new individuals. Genotyping error rates were estimated using Pedant (Johnson and Haydon, 2007), with 10 000 search steps. Since the software only compares two replicates at a time, we carried out all possible pairwise comparisons and then averaged the results.

2.4. Data analysis

To estimate vole abundance (*N*), we considered each seasonal survey as a single sampling event and used 'continuous-occasion' closed-population CMR models allowing for multiple captures of the same individual within each survey. Specifically, we used the *capwire* estimator based on urn models (Miller et al., 2005; Pennell et al., 2013), considering two alternative formulations: the equal capture probability model (ECM) and the two innate rates model (TIRM). In ECM, all individuals are considered equally likely to be captured on each survey, while in TRIM there is a mixture of two types of individuals with different capture probabilities. A likelihood-ratio test was used to evaluate the fit of both models and determine the best fit (Miller et al., 2005). We used a parametric bootstrap test with 1000 samples to generate the 95% confidence interval for population estimates of the best model for each season. Both ECM and TRIM were fitted and compared in the package *capwire* (version 1.1.4) (Pennell et al., 2013) for R (version 3.3.2) (R Core Team, 2016). For comparison purposes, we also used Chao's lower bound estimator assuming individual heterogeneity in capture probabilities (M_h-Chao) (Chao, 1989). Although the M_h-Chao estimator assumes different capture occasions, it uses only the capture frequency, so it may be applied to our data (Miller et al., 2005). This estimator is thought to outperform capwire for large datasets (N > 200) (Miller et al., 2005), such as that used in this study (see Results). For this, we used the R package Rcapture (version 1.4.2) (Baillargeon and Rivest, 2007).

To estimate monthly recapture (p) and survival (ϕ) probabilities we used the Cormark-Jolly-Seber (CJS) open-population model approach (Lebreton et al., 1992), implemented in *RMark* (v 2.2.4; Laake, 2013), an R interface for software package MARK (White and Burnham, 1999). For this, we first collapsed within-season capture histories for each genotyped individual into a single value (0/1), denoting whether it was identified or not at each season (McCrea and Morgan, 2015). We then tested the goodness-of-fit on a fully time-dependent CJS model (McCrea and Morgan, 2015) using the *R2ucare* (v 1.0.0; Gimenez et al., 2017), which suggested a good fit of the data (χ^2=2.34, p-value=0.311). Potential predictors of CJS parameters included the effects of genotyping success on p; the effects of time, sex, and patch area on both p and ϕ; and the effects of patch isolation, patch permanence, presence of road-verges, presence of permanent water-bodies, detection of southern water voles, distance to the nearest agricultural area (vegetable gardens, orchards, or ploughed fields), and distance to the nearest urban area (e.g. houses, social areas, buildings) on ϕ (see full description and rationale of covariates in Table

1). Environmental covariates were specified at the individual level, considering the moment of first capture.

Before model building and selection, we assessed the correlations among predictors of *p* and among predictors of ϕ, retaining in the analyses only the predictors with correlation coefficients <0.30 (i.e. low correlations; Graham, 2003). Such a conservative criterion was chosen because in CJS models, as the number of predictors increases, so will the number of model parameters, and hence the number of possible models under evaluation, which decreases the ability to distinguish between informative and spurious variables (Doherty et al., 2012). Among the potential predictors of *p*, we found a negative correlation between genotyping success and patch area (Table A2, Appendix A). Because *p* should be most critically affected by variables more directly related to the sampling design, we retained genotyping success in the analyses to the detriment of patch area. As regards to potential predictors of ϕ, we found some degree of multicollinearity among the presence of road-verges, patch area, patch permanence, and patch isolation (Table A2, Appendix), suggesting an association of road verge habitats to larger, more stable, and less isolated patches. Because the presence of road-verges was considered to provide a reliable descriptor of local environmental variations directly linked to human land-use activity, this variable was carried out to the CJS modelling procedure. We also retained the covariate distance to agricultural fields instead of the distance to urban areas (positively cross-correlated; Table A2, Appendix A), as agricultural land-use was predominant, and potentially most relevant land-use in the study area. Sex, presence of water, and detection of water voles were only weakly correlated to other predictors, and were therefore also retained in the analyses (Table A2, Appendix A).

We then evaluated a set of CJS models translating different combinations of hypotheses regarding uncorrelated factors possibly affecting *p* and ϕ (Lebreton et al., 1992). Given the relatively large number of possible models considering all possible combinations of main effects in both *p* and ϕ (i.e., 512 models), we combined the most plausible submodels found separately for each parameter (Bromaghin et al., 2013). Plausible submodels for both *p* and ϕ were identified in each case, by first building and ranking all possible submodels defined by all possible additive combinations of main effects (three in the case of *p* and six in the case of ϕ). This resulted in eight submodels describing *p*, and 64 submodels describing ϕ. For the final set of candidate models to be evaluated, we retained in each case the set of submodels with a difference of Akaike's Information Criteria corrected for small sample size (ΔAIC_c) smaller than 2

relative to the respective top-ranked submodel, regarded as the most supported (Burnham and Anderson, 2002). This plausible combination approach reduced the number of candidate models to about 3% (n=15) in relation to the all possible combinations strategy (see Results), thus reducing the potential incidence of spurious results (Doherty et al., 2012). Finally, from the most supported models (i.e. ΔAICc<2 relative to the top-ranking model) within this final set of 15 candidate models, we discarded those including uninformative covariates [i.e. with 85% confidence intervals of effect size estimates including zero; Arnold (2010)]. This resulted in the selection of one single best model, from which we estimated the predicted monthly p and ϕ relative to each informative covariate included in that model.

3. Results

The amount of suitable habitat increased from the early wet season (EWS; 36 ha) to the late wet season (LWS; 46 ha), and declined both in the early dry season (EDS; 41 ha), and in the late dry season (LDS; 29 ha) (Fig. 1). The percentage of occupied patches was of 45% (n= 131) in EWS, 51% (n=138) in LWS, 61% (n= 137) in EDS, and 54% (n=149) in LDS (Fig. 1).

We collected a total of 2 711 faecal samples (mean±SE per season = 678±54), of which 48.4% (n=1312; 328±24 per season) were selected for DNA extraction and genotyping. Average genotyping success rate was 33.9% (444 samples), with the highest values obtained for samples collected in EDS (42.4%, n=153), followed by EWS (38.4%, n=140), LWS (26.8%, n=87), and LDS (24.4%, n=64). Overall genotyping errors were low (dropout rate: 0.68-2.8%; false allele rate: 0-0.18%; Table A1, Appendix A), with higher genotyping errors recorded in the seasons with lower genotyping success. From the randomly selected 23% of samples that failed amplification (i.e. 163 out of 704 samples), about 86% were identified as Cabrera voles, while the others belonged to other rodent species (7.3%) or were contaminated with human or ungulate DNA (6.7%). Another 164 samples were also contaminated despite being successfully amplified. The 444 samples that were successfully amplified and were not contaminated were assigned to a total of 307 individuals, with 81 (EWS), 77 (LWS), 122 (EDS), and 64 (LDS) individuals per season. From the 137 recaptures identified across all surveys, 120 (87.6%) were in the same habitat patch of the previous (re)capture. The mean seasonal sex-ratio was even (M:F = 1.08), with no marked seasonal changes (between 1.03 and 1.13).

The likelihood-ratio tests indicated that ECM was more supported than TRIM for estimating abundances in all seasons (p> 0.16). Abundances estimated by ECM (range: 116-353) were very

311 similar to those obtained using M_h-Chao (range: 125-370). There was an over 4-fold increase in
312 vole abundance from EWS to LWS, with little change through to the EDS, followed by about a 2-
313 fold decrease until the LDS (Fig. 2). Except for EWS, abundance estimates derived from both
314 ECM and M_h-Chao had relatively wide confidence intervals.

315 The PC model selection approach resulted in the retention of five recapture probability
316 submodels and three survival probability submodels (Table 2). After applying the model
317 selection procedure on the final set of 15 plausible models and excluding models with
318 uninformative covariates, the most supported model retained no covariates affecting recapture
319 and included four covariates affecting survival (Table 3) Monthly recapture probability was
320 estimated as 0.54 (0.20-0.85 CI 95%), while monthly survival was estimated as 0.52 (0.39-0.65 CI
321 95%), being 1.5-times higher in males than in females (Fig. 3A). Survival was also affected by
322 habitat conditions, being 2-times higher on road-verges than elsewhere (Fig. 3B), 1.5-times
323 higher in the presence of (or bordered by) water-bodies (Fig. 3C), and 2-times higher 300m
324 away from agricultural areas (Fig. 3D).

325 **4. Discussion**

326 We demonstrated for the first time the usefulness of large-scale genetic non-invasive sampling
327 combined with capture-mark-recapture methods to estimate and identify the factors affecting
328 small mammal demographic parameters and infer their population dynamics. Using the near-
329 threatened Cabrera vole in Mediterranean farmland, we showed that our approach provides
330 key information to improve conservation planning of elusive small mammals, especially those
331 threatened by human activities and that are difficult to sample using traditional methods (Cheng
332 et al., 2017).

333 Our approach showed that Cabrera vole abundance varied greatly across an annual cycle,
334 confirming a large increase in vole numbers along the wet season (67%), and a substantial
335 decline through the dry season (44%), as reported elsewhere from live-trapping data collected
336 at more confined scales (Fernández-Salvador et al., 2005b; Rosário, 2012). These changes
337 seemed largely unrelated to seasonal genotyping success, which lowered by the end of the wet
338 season and by the end of the dry season, likely due to increased DNA degradation under higher
339 rainfall and sunlight exposure, respectively (Santini et al., 2007). Seasonal variation in vole
340 numbers was consistent with the described breeding period for this species, which suggests a
341 lower activity of individuals during the dry season, when reproduction may even cease

completely (Fernández-Salvador et al., 2005b; Pita et al., 2006). Comparable seasonal fluctuations have also been found in other rodent species inhabiting highly seasonal Mediterranean environments (Cohen-Shlagman et al., 1984; Gomez et al., 2016), as well as in other Iberian endemic herbivores, like the Iberian rabbit (*Oryctolagus cuniculus algirus*) (Gonçalves et al., 2002). This pattern is thought to be related to variations in habitat and food availability, which in our study area are generally reduced during the dry season (Pita et al., 2014). This was reflected in our data by the 37% decrease in habitat availability from the end of the wet season to the end of the dry season, in accordance with the natural seasonal variation in climate, and was also observed for other voles in intensively managed Mediterranean farmlands [e.g. common vole *Microtus arvalis*; Rodríguez-Pastor et al. (2016)]. Pedigree and sibship analyses indicated that our gNIS was able to detect animals from different generations within each season (Ferreira et al., unpublished data), suggesting no serious bias towards any particular age-class of the population. While we acknowledge that the two-month duration of our seasonal surveys warrants some caution regarding abundance estimates obtained within a closed CMR framework, these estimates likely describe the broad patterns of seasonal population change at the surveyed landscape and its surroundings (Boulanger and McLellan, 2001). Therefore, our study suggests that estimating vole population size based on gNIS and CMR modelling allows for the drawing of inferences on abundance variation across time.

Estimates of apparent survival of Cabrera voles based on gNIS in Mediterranean farmland were relatively low (0.39-0.65 CI 95%), though still within the range usually observed in other semi-fossorial *Microtus* species living in agricultural landscapes across different geographical regions. For instance, the survival of *M. agrestis* in field margins in fragmented farmland from northeast Scotland ranged between 0.42 and 0.69 (Renwick and Lambin, 2011), while that of *M. arvalis* in agricultural landscapes from central western France varied between 0.22-0.69 (Bonnet et al., 2013). Similar survival rates (0.25-0.64) were also reported for *M. pennsylvanicus* in forage crops from Illinois (Getz et al., 2007). Surprisingly, despite the presumed lower habitat quality in our study area during the dry season, and the observed temporal variation in Cabrera vole abundance, survival was virtually constant across time. Variation in population abundance, despite constant survival rates, suggests that population recruitment (newborn and immigrants) increased during the wet season (when abundance increased), and decreased during the dry season, probably until it became insufficient to compensate the low survival rates, thereby resulting in pronounced decreases in vole numbers. Although we did not assess recruitment

374 explicitly and our gNIS approach inherently prevents the identification of individuals' age, the
375 observed seasonal variations in abundance agrees with the monthly fluctuations in recruitment
376 rates observed in Cabrera vole populations from other Mediterranean areas (Fernández-
377 Salvador et al., 2005b), as well as in other vole species from Mediterranean-like climates
378 (Cockburn and Lidicker, 1983; Cohen-Shlagman et al., 1984; Paradis and Guédon, 1993).

379 Survival was however not constant across space. Voles detected closest to farmed areas showed
380 a 53% lower survival probability than voles captured furthest away, suggesting a negative
381 impact of agriculture management on voles. Agriculture activities in this area are highly
382 dynamic, involving for instance the conversion of fallow areas to farmed areas, resulting in the
383 destruction of habitat-patches. While the putative negative effects of agricultural intensification
384 on Cabrera vole populations have been widely suggested (Fernández-Salvador et al., 2005b;
385 2005a; Pita et al., 2006), our study provides the first quantitative evidence that the distance of
386 grass-rich fragments occupied by voles to unsuitable farmed habitat affects individual survival
387 probability. Further studies analysing fecundity variation across space and time are however
388 needed to fully understand how such effect impacts overall population persistence in farmland
389 areas. According to our initial predictions, voles captured in road-verge habitats showed higher
390 survival probabilities than voles captured elsewhere. While road-verge habitats can be viewed
391 as suboptimal for voles (Santos et al., 2007) it is likely that in intensively-used Mediterranean
392 farmland, these habitats provide refuges for the species, as their vegetation is often left
393 undisturbed for relatively long-time periods compared to surrounding fields (Ruiz-Capillas et al.,
394 2013). This is supported by the association of road-verge habitats to larger and more stable
395 habitats in our study area, as well as by the low traffic volume associated to the roads. The value
396 of road-verges as refuges for small mammals in farmlands has also been noted in other
397 Mediterranean environments with varying levels of land management (Ascensão et al., 2012;
398 Ruiz-Capillas et al., 2013; Sabino-Marques and Mira, 2011), being generally attributed to
399 increased habitat quality, and/or to predation release effects, in the case of roads with more
400 intensive traffic (Rytwinski and Fahrig, 2007).

401 The positive effects of the presence of permanent water-bodies within or bordering habitat-
402 patches on voles' survival was in accordance to the prediction that damper habitats provide
403 better resources for Cabrera voles (Pita et al., 2011). This may be associated with the presence
404 of fresh green vegetation providing high-quality food and shelter across the dry landscape
405 (Santos et al., 2007). While the presumed superior competitor water vole also prefers habitats

406 with dense and wet vegetation, and may affect Cabrera voles' occupancy patterns (Pita et al.,
407 2016), we found no evidence for inter-specific effects on Cabrera voles' survival. It is also
408 interesting to note that, despite their influence in shaping Cabrera voles' occupancy dynamics
409 and abundance (Pita et al., 2007), patch size and isolation were poor predictors of individual
410 survival. This has also been reported in other small mammal species, for which habitat quality
411 was also a better surrogate for survival than patch size (Mortelliti et al., 2014).

412 Besides environmental factors, and contrary to our expectations based on the predominantly
413 monogamous mating systems of Cabrera voles (Pita et al., 2014), apparent survival was also
414 related to sex, with support for higher survival in males. In the case of monogamous species that
415 are not sexually dimorphic and with both males and females sharing parental care, survival
416 generally tends to be similar across sexes or in some cases male-biased, thus highlighting an
417 interesting exception to the general rule of male-biased mortality, typical in polygynous
418 mammals (Clutton-Brock and Isvaran, 2007). Higher male survival in monogamous species may
419 result from reduced competition among males compared to polygynous animals, and the fact
420 that even in the presence of parental care, the costs of raising off-spring are likely to be endured
421 primarily by lactating females (Clutton-Brock and Isvaran, 2007; Lukas and Clutton-Brock, 2013).
422 Testing this hypothesis explicitly would require information on individuals' reproductive
423 condition and age, which are not obtainable from gNIS approaches such as ours. However,
424 because male-biased survival did not result in any male-biased sex-ratios, and apparent survival
425 is the product of true survival and site fidelity (Sandercock, 2006), we cannot rule out the
426 possibility that the observed male-biased survival could also reflect a female-biased dispersal (or
427 permanent emigration), which is also common in monogamous species (Mabry et al., 2013).
428 Clearly, more studies are needed to fully elucidate on possible male-biased survival and/or
429 female-biased dispersal in the Cabrera vole. This warrants the recommendation that future
430 gNIS-based studies aiming to assess survival of elusive species, should combine other methods
431 providing information on individual reproductive status and age, so as to improve inferences on
432 their population dynamics.

433

434 **5. Conclusions**

435 Overall, our study provides empirical evidence that gNIS is a useful tool to monitor small
436 mammal population parameters, and to identify management actions that may prove necessary

to maintain their populations. Regarding the Cabrera vole, our results support the idea that conservation measures aimed to increase its survival in Mediterranean farmland, should promote low intensity agricultural management near occupied patches (encompassing longer fallow periods, low-disturbed margins, and high density of permanent water-bodies), in order to allow the continued existence of suitable habitats for the species, particularly during the stressful dry-season, when habitat availability is lower. We consider that our approach may be applied to other elusive small or medium mammals requiring conservation action, but for which informative demographic data across large spatial and temporal scales are still lacking.

Author contributions

Conception (PCA, PB, AM, RP); Design (XL, PCA, PB, AM, RP); Data Collection (APF, IL, CF, JP, HSM, SB); Data Analysis (APF, CF, RP); Writing (APF, RP); Revision (APF, CF, JP, PCA, HSM, SB, XL, PB, FM, AM, RP).

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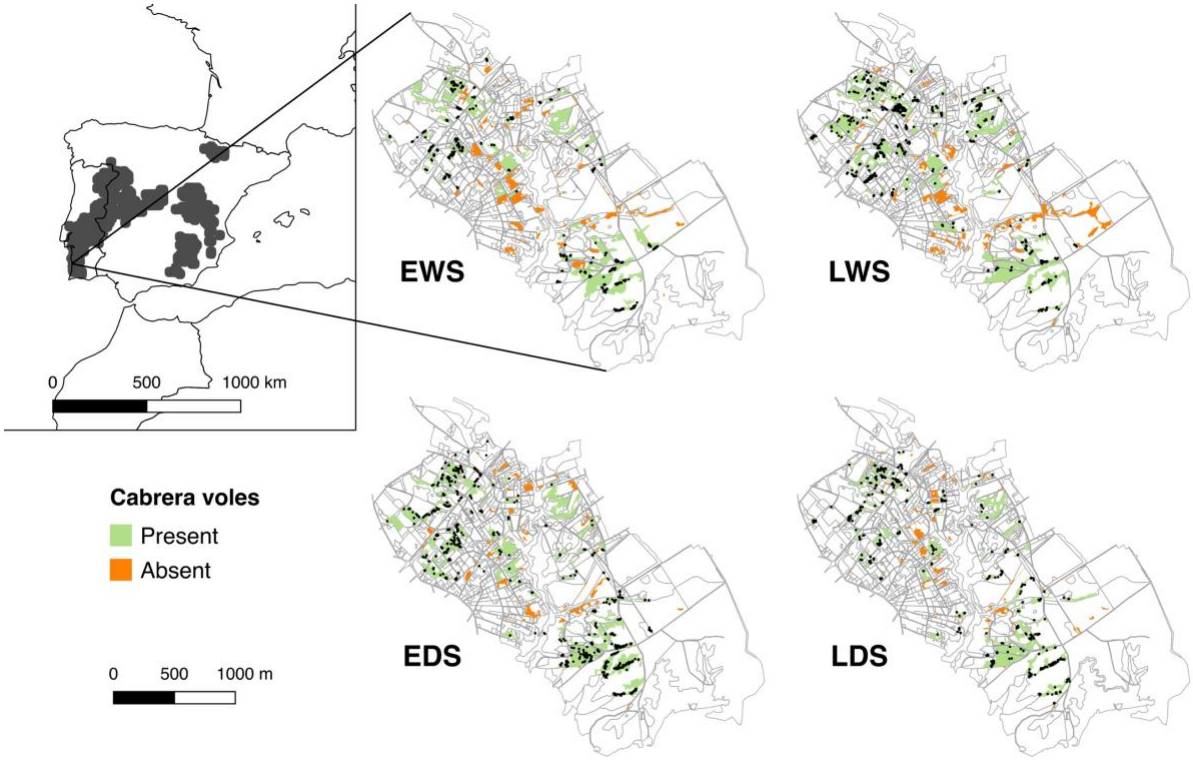

Fig. 1 – On the upper left, Cabrera vole distribution (in dark-grey, adapted from Mestre et al., 2015) and study area location. On the right, four seasonal surveys made in a 461.8 ha area within the coastal plateau of SW Portugal. EWS - early wet season, November and December 2013; LWS - late wet season, February and March 2014; EDS - early dry season, May and June 2014; LDS - late dry season, September and October 2014. Coloured polygons represent identified suitable habitat-patches for voles. Black dots represent fresh-faeces samples collected for genetic analyses.

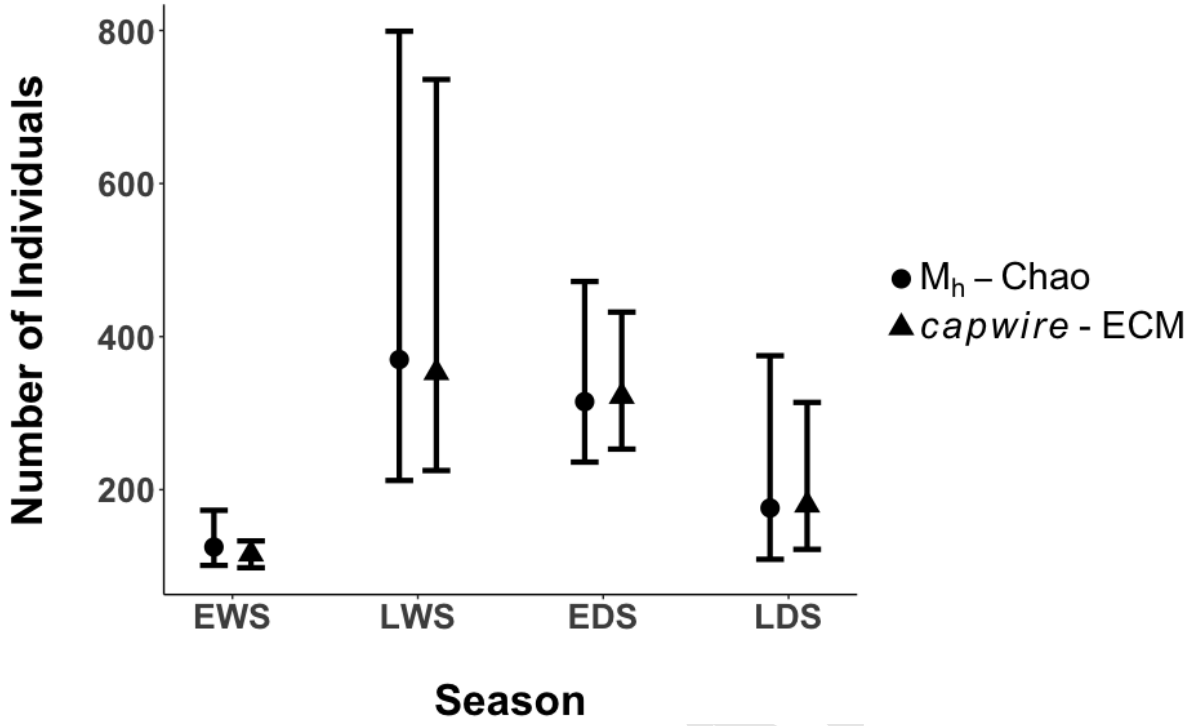

Fig. 2 – Seasonal population abundance of Cabrera voles in a Mediterranean farmland, based on genetic non-invasive sample, estimated using the M_h-Chao estimator (Chao) and the *capwire*'s equal capture probability model (ECM). EWS - early wet season, November and December 2013; LWS - late wet season, February and March 2014; EDS - early dry season, May and June 2014; LDS - late dry season, September and October 2014. Bars indicate 95% confidence intervals.

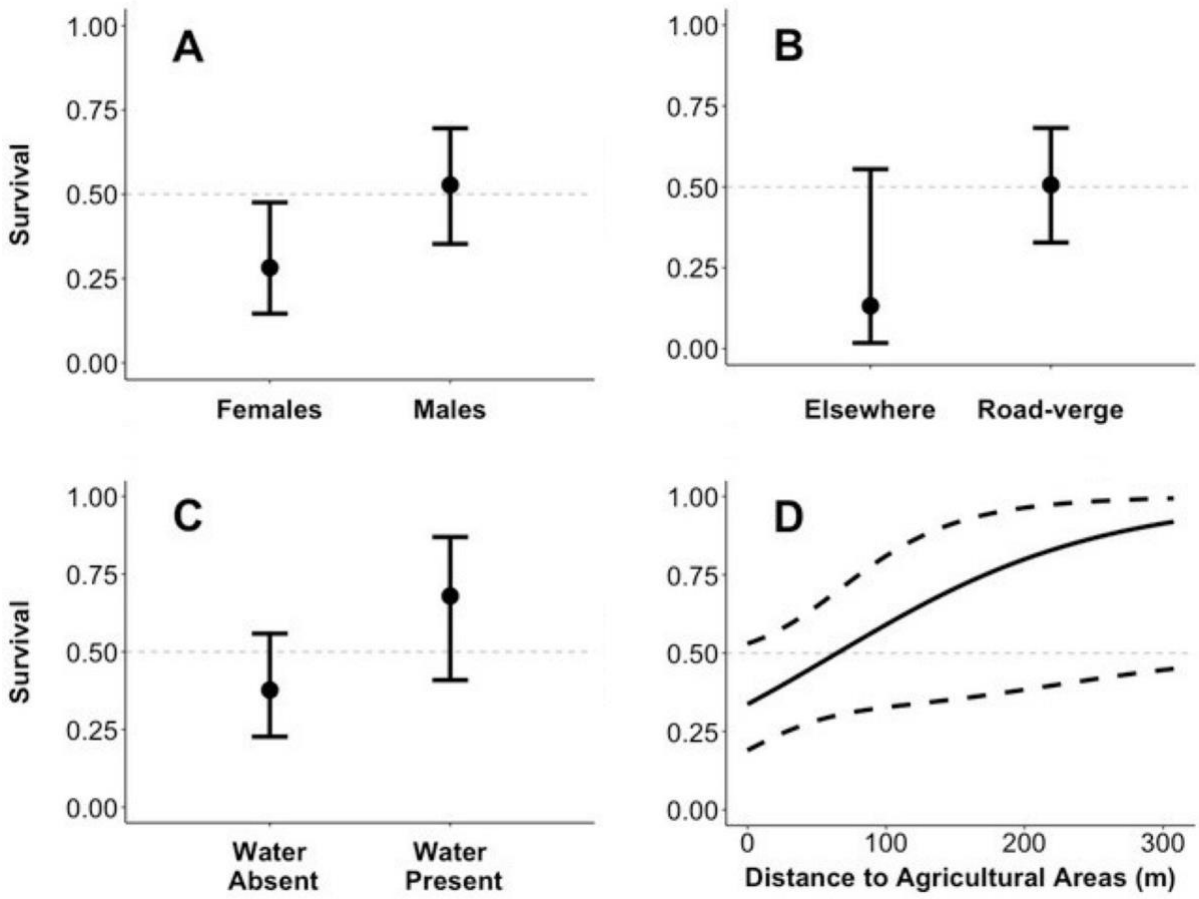

Fig. 3 – Apparent survival of Cabrera vole relative to sex (A); road verges (B); presence of water (C); and distance to agricultural areas (D), based in genetic non-invasive sampling in a Mediterranean farmland, during the period between November 2013 and October 2014. Black bars and dashed lines represent 95% confidence intervals. Grey dashed line represents average monthly apparent survival (0.52).

Table 1 – Definition and summary statistics of covariates used in Cormack-Jolly-Seber (CJS) models to estimate recapture probabilities (p) and survival (ϕ) of the Cabrera vole in a Mediterranean farmland landscape of SW Portugal. Underlying hypotheses regarding covariate effects tested on p and ϕ are also presented. All environmental covariates were measured for each individual at the time of first capture (see text). * indicates variables selected for the CJS modelling procedure (see *Data analyses*).

Covariate Code	Description	Type	N	Mean ± SE [range]	Parameter	Underlying hypothesis (based on Pita et al., 2014, except where indicated)
*GenSuccess**	Mean genotyping success of analysed samples in each patch	Proportion (0-1)	307	0.5 ± 0.01 [0.1 – 1]	p	p is higher in patches where genotyping success was higher
*Time**	Transition between seasons	Categorical (EWS-LWS; LWS-EDS; EDS-LDS)	307	-	$p\ \phi$	p and ϕ differ among seasons, being greater in spring when animals are more active, and habitat and food availability are greater; and lower in the summer when habitat and food resources are scarcer, and animals are less active.
*Sex**	Sex of individuals	Categorical (M/F)	M: 152 F: 155	-	$p\ \phi$	p is similar between sexes, as home-ranges vary little between males and females, and both sexes are expected to equally mark their territories. ϕ is similar between sexes, as expected for monogamous mammals (Clutton-Brock and Isvaran, 2007)
PArea	Area of the patch where animals were captured (m^2)	Continuous (> 0)	307	9834.0 ± 658.93 [106.2–51764.0]	$p\ \phi$	p decreases with patch area, as animals tend to concentrate their territories within particular areas of habitat-patches. ϕ increases with patch area, as larger patches should provide more habitat and food resources.
PIsolation	Mean distance from the patch to the 3 nearest patches (m)	Continuous (> 0)	307	34.7 ± 2.06 [3.4–187.9]	ϕ	ϕ decreases with increasing patch Isolation, due to increased mortality during movement attempts between patches further apart from each other.
PPermanence	Permanence of the patch in the following seasons	Categorical (0/1)	0: 73 1: 234		ϕ	ϕ is lower if patch disappears in the following seasons
*RoadVerge**	Binary coding of whether the patch is a roadside habitat (<10m from a road)	Categorical (0/1)	0: 66 1: 241	-	ϕ	ϕ is higher in roadside habitats, as these are usually less disturbed by farming operations.
*Water**	Presence of a permanent water-body within or at the border of the patch	Categorical (0/1)	0: 278 1: 29	-	ϕ	ϕ is higher where water is present, as the species prefers wet vegetation providing both refuge and fresh food
*Arvicola**	Occurrence of *Arvicola sapidus* in the patch	Categorical (0/1)	0: 102 1: 205	-	ϕ	ϕ is lower where the competitively superior *A. sapidus* is present
AgroDist	Distance from patch to the nearest agricultural area (m)	Continuous (≥ 0)	307	27.9 ± 3.20 [0–248.9]	ϕ	ϕ is lower in patches closer to agricultural fields, as these are associated to increased disturbance resulting from farming operations.
UrbDist	Distance from the patch to nearest urban area (m)	Continuous (≥ 0)	307	36.9 ± 3.07 [0–247.6]	ϕ	ϕ is lower in patches closer to urban areas, as these are associated to increased human disturbance and/or predation by domestic cats and dogs.

Table 2 – Set of best plausible models (with a difference of Akaike's Information Criteria corrected for small sample size < 2) obtained from the set of 8 submodels describing *p* (keeping ϕ fixed) and 64 submodels describing ϕ (keeping *p* fixed).

Varying *p*, fixed ϕ	**AIC_c**	**k**
~1	178.1	9
~Time	178.4	11
~Sex + Time	179.8	12
~Sex	179.9	10
~GenSuccess	179.9	10
Varying ϕ, fixed *p*		
~RoadVerge + AgroDist + Sex + Water	178.1	10
~RoadVerge + AgroDist + Sex + Water + Time	179.9	12
~RoadVerge + AgroDist + Sex + Water + Arvicola	180.0	11

AIC_c –Akaike's Information Criteria corrected for small sample size; k – degrees of freedom; ϕ – apparent survival probability; *p* – recapture probability.

Table 3 – Ordered set of best ranked plausible models (n = 15), obtained by fitting models separately for recapture (p) and apparent survival (ϕ) probabilities (more details in the Material and Methods section and in Bromaghin et al., 2013). Among the three most supported models (with $\Delta AIC_c < 2$), the top-ranked model (in bold, $AIC_c = 172.24$) was the only one not including uninformative covariates, being thus retained as the best model. See definition of covariates in Table 1.

ϕ	p	ΔAIC_c	w_i	k
~AgroDist + RoadVerge + Sex + Water	**~1**	**0**	**0.29**	**6**
~AgroDist + RoadVerge + Sex + Water	~Sex	1.76	0.12	7
~AgroDist + RoadVerge + Sex + Water	~GenSuccess	1.80	0.12	7
~AgroDist + RoadVerge + Sex + Water + Arvicola	~1	2.05	0.10	7
~AgroDist + RoadVerge + Sex + Water	~Time	3.07	0.06	8
~AgroDist + RoadVerge + Sex + Water + Arvicola	~Sex	3.74	0.04	8
~AgroDist + RoadVerge + Sex + Water + Time	~1	3.83	0.04	8
~AgroDist + RoadVerge + Sex + Water + Arvicola	~GenSuccess	3.84	0.04	8
~AgroDist + RoadVerge + Sex + Water	~Sex + Time	3.89	0.04	9
~AgroDist + RoadVerge + Sex + Water + Time	~Time	4.00	0.04	10
~AgroDist + RoadVerge + Sex + Water + Arvicola	~Time	5.05	0.02	9
~AgroDist + RoadVerge + Sex + Water + Time	~Sex + Time	5.43	0.02	11
~AgroDist + RoadVerge + Sex + Water + Time	~GenSuccess	5.60	0.02	9
~AgroDist + RoadVerge + Sex + Water + Time	~Sex	5.65	0.02	9
~AgroDist + RoadVerge + Sex + Water + Arvicola	~Sex + Time	5.83	0.02	10

ΔAIC_c – Difference of Akaike's Information Criteria corrected for small sample size; w_i – model relative Akaike weight; k – degrees of freedom.