ORIGINAL ARTICLE**Contrasting demographic trends and asymmetric migration rates in a spatially structured amphibian population****Running title:** Integrative monitoring of a frog metapopulation

Berta Capellà Marzo, Gregorio Sánchez-Montes, Iñigo Martínez-Solano*

Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales, CSIC, c/ José Gutiérrez Abascal, 2, 28006 Madrid, Spain

* Correspondence: Iñigo Martínez-Solano,

e-mail: inigomsolano@mncn.csic.es

ABSTRACT

Natural populations often persist at the landscape scale as metapopulations, with breeding units (subpopulations) experiencing temporal extinction and recolonization events. Important parameters to forecast population viability in these systems include the ratio of the effective number of breeders (N_b) to the total number of adults (N_a) and migration rates among subpopulations. Here we present the results of a 10-year integrative monitoring program of a metapopulation of the Iberian green frog (*Pelophylax perezi*) in central Spain. We characterized population dynamics at two main breeding ponds (Gravera and Laguna) using capture-mark-

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/1749-4877.12449](#).

This article is protected by copyright. All rights reserved.

recapture data to estimate N_a in each breeding season, and multilocus genotypes to estimate the effective population size (N_e), N_b , individual breeding success and migration rates. Both ponds experienced population decline after a dry season, with Gravera subsequently recovering and Laguna suffering a bottleneck associated with genetic impoverishment. In this subpopulation, average allelic richness and private alleles decreased from 2010 (10.87 and 1.67, respectively) to 2018 (8.0 and 0.20). The N_b/N_a ratio in Laguna in 2018 was twice as high (0.95) than in Gravera (0.41) or in pre-bottleneck Laguna (0.50), suggesting plasticity or genetic compensation through increased individual breeding success. Migration rates were asymmetric between ponds, with a stronger contribution from Gravera to Laguna (29.9% vs. 16.2% in the opposite direction) that may result in a rescue effect. This study emphasizes the importance of integrative demographic approaches for the monitoring of natural populations based on a better understanding of their spatio-temporal dynamics, which provides valuable information for conservation actions.

Key words: Bottleneck, capture-mark-recapture, genetic monitoring, metapopulation, N_b/N_a ratio.

1. INTRODUCTION

Many populations persist at the landscape scale as metapopulations or spatially structured populations, composed of a network of breeding units (subpopulations) connected by gene flow. In this metapopulation framework, breeding units can be classified as sources or sinks, with positive or negative net population growth rates, respectively. The role of subpopulations as sources or sinks can vary through time (Dias 1996; Thomas & Kunin 1999). Classical metapopulation dynamics are characterized by temporal colonization-extinction events, where subpopulations can become locally extinct (with higher probability in the case of sinks) due to demographic stochasticity or catastrophic events, with subsequent recolonization by migrants from other subpopulations (usually from a source: rescue effect, Taylor & Harrison 1997; González *et al.* 1998; Hufbauer *et al.* 2015). The probability of persistence of subpopulations is mainly a function of i) their size, with larger populations being more likely to persist in the long term, and ii) their connectivity with other subpopulations, with highly connected units showing greater capacity of balancing out local extinctions through rescue effects via migration (Fahrig & Merriam 1985; Taylor & Harrison 1997; Shtilerman & Stone

2015). Thus, accurate characterization of metapopulation dynamics (including identification of sources and sinks and inferring patterns of interconnectivity) requires estimates of population sizes and dispersal rates across subpopulations.

Natural populations often experience large and irregular fluctuations in size that can be caused by intra- and interspecific interactions, such as competition or predation, or by exogenous abiotic factors, like climatic conditions (Levins 1969). In the long term, small and isolated populations are more likely to be affected by these fluctuations, which can lead to severe reductions in population size (bottlenecks) (Wilfried & Bürger 1992; Habel & Zachos 2013). Bottlenecks can lead to an impoverishment of the genetic pool, increasing the probability of inbreeding depression (Lacy 1987; Xenikoudakis *et al.* 2015). The loss of genetic diversity reduces the ability of the population to adapt to environmental changes and increases its extinction risk (Lande & Shannon 1996; Sonsthagen *et al.* 2017). In metapopulations, immigration of individuals to a post-bottlenecked subpopulation is expected to increase genetic diversity and, accordingly, reduce its extinction risk ('genetic rescue'; Hufbauer *et al.* 2015). In addition, rescue effects can occur even if the new immigrants do not increase genetic diversity (i.e., through demographic rescue). Thus, dispersal among subpopulations can mitigate the negative effects of demographic fluctuations on genetic diversity (McEachern *et al.* 2011; Jangjoo *et al.* 2016), emphasizing the importance of connectivity between subpopulations for the long-term persistence of metapopulations. Connectivity patterns between subpopulations are usually asymmetric, with net positive or negative emigration/immigration ratios (Bode *et al.* 2008). From a conservation perspective, identifying breeding units that are more important for the long-term survival of the metapopulation is paramount to predict their fate in the face of threats like habitat fragmentation and loss or changing climatic regimes (Bettini *et al.* 2015; Furrer & Pasinelli 2015; Hanski *et al.* 2017). Some characteristics of breeding units with a higher leverage on metapopulation resilience include larger and demographically more stable subpopulations capable of producing migrants for other units of the metapopulation ("source" vs. "sink" subpopulations, respectively).

The effective population size (N_e) and the population size (N) are two key demographic parameters that, in addition to providing important insights on the ecology and evolution of (meta)populations (Palstra & Fraser 2012), can help characterize their spatio-temporal dynamics. The effective size (N_e) is the number of individuals in a Wright-Fisher population (constant size, discrete generations, random mating and allele frequencies in Hardy-Weinberg equilibrium) with the same genetic properties (in terms of random genetic drift) as the target population (Fisher 1930; Wright 1931). The parameter N_e is often used to determine the amount of inbreeding and the loss of genetic variability in a finite population, so it is a crucial

parameter to consider in conservation (Charlesworth 2009; Hare *et al.* 2011). On the other hand, different definitions have been used to approximate N , like the census size (N_c), the total number of individuals present in the target population, or the number of adults (N_a) (Nunney & Elam 1994; Frankham 1995). The population size governs ecological processes (such as competition, predation and population growth rate), whereas evolutionary processes (including the rates of genetic drift and loss of genetic variability) depend on the effective size (Waples *et al.* 2014). The relationship between these two parameters is expressed by the ratio N_e/N , which is a key determinant of the adaptive potential and stability of natural populations (Frankham 1995; Palstra & Fraser 2012). The effective size is generally lower than the population size because of the variance in the genetic contribution of individuals to the next generation (Frankham 1995). A high N_e/N ratio (close to a value of one) indicates that most adults in the population contribute to the next generation, whereas a low ratio implies a high variance in breeding success among adult individuals, leading to a potentially faster genetic impoverishment compared to populations with higher N_e/N ratios (Ferchaud *et al.* 2016; Sánchez-Montes *et al.* 2017b).

The study of N_e is often complicated in iteroparous species with overlapping generations (Lee *et al.* 2011). For these species, the effective number of breeders in a reproductive cycle (N_b) can be used to estimate the ability of the population to maintain genetic diversity. The parameter N_b is easier to estimate because it only requires data from a single-cohort offspring sample (one breeding season; Beebee 2009). In contrast, mixed-age samples are required to estimate N_e , which corresponds to the effective size in a complete generation (Waples *et al.* 2013, 2014). Thus, the N_b/N_a ratio can be considered an approximation of the N_e/N ratio as applied to a single breeding season (Sánchez-Montes *et al.*, 2017b). Both N_e and N_b coupled with the N_b/N_a ratio provide good proxies for subpopulation sizes in spatially structured populations, with higher values expected to be associated with higher demographic stability. Complementing estimates of demographic parameters like N_e , N_b , N_a and their ratios with inference of dispersal rates across subpopulations allows obtaining valuable insights about metapopulation dynamics to inform management actions, including the identification of key nodes and their relative probability of persistence.

Pond-breeding amphibians can be studied as metapopulations, where each breeding pond corresponds to a discrete breeding unit or subpopulation subject to local extinction-recolonization dynamics, surrounded by a heterogeneous terrestrial habitat matrix (Allentoft & Brien 2010; Griffiths *et al.* 2010). Amphibian populations are generally known to experience strong demographic fluctuations, because of their high sensitivity to environmental changes. For example, seasonal variation in patterns of rainfall can have large impacts on the

hydroperiod of temporary breeding ponds, resulting in high inter-annual variance in larval survival until metamorphosis (Buckley & Jetz 2007; Cayuela 2016). Under a metapopulation perspective, connectivity between ponds and their rates of exchange of migrants are key determinants for the long-term persistence of amphibian populations at both the local (subpopulation) and landscape (metapopulation) level. Understanding the causes of turnover in pond occupation and studying inter-pond migration rates thus has important implications for predicting the future of metapopulations.

Here we present the results of a 10-year (2010-2019) demographic monitoring program of a metapopulation of the Iberian green frog (*Pelophylax perezi* (López-Seoane 1885)) in central Spain, characterizing temporal changes in population size and quantifying patterns of connectivity in the two main breeding sites in the study area. We use a novel, integrative approach combining capture-mark-recapture (CMR) data and multilocus genotypes to estimate the number of adults (N_a) for each breeding season and to quantify migration rates between breeding sites. In addition, we estimate the effective population size (N_e) of each subpopulation and compare the effective number of breeders (N_b) and the N_b/N_a ratio at two time points (2010 and 2018) in order to assess the effect of an observed population decline on genetic diversity. Finally, we estimate the average breeding success of both males and females at the two breeding sites and discuss the implications of observed variation in this parameter for the long-term maintenance of genetic diversity in the metapopulation.

2. MATERIALS AND METHODS

2.1. Study Area

The study area is located between the towns of La Cabrera and Valdemanco (Comunidad de Madrid, Spain), in the foothills of La Cabrera ridge, in Sierra de Guadarrama (see Sánchez-Montes & Martínez-Solano 2011). There are two main temporary ponds that are used for breeding by a rich amphibian community, including the Iberian ribbed newt *Pleurodeles waltl* Michahelles 1830, the marbled newt *Triturus marmoratus* (Latreille 1800), the Iberian midwife toad *Alytes cisternasii* Boscá 1879, the Iberian spadefoot toad *Pelobates cultripes* (Cuvier 1829), the natterjack toad *Epidalea calamita* (Laurenti 1768), the Iberian tree frog *Hyla molleri* Bedriaga 1889 and *Pelophylax perezi*.

The largest pond is Laguna de Valdemanco (hereforth referred to as Laguna: 40.8531°N, 3.6449°W, 1054 m a.s.l.), a temporal water body with a maximum surface area of 12,800 m² and 1 m of maximum depth. In the centre there are patches of lakeshore bulrush (*Schoenoplectus lacustris*), and the rest of the pond is covered by aquatic macrophytes. Predominant vegetation

in the area includes gum rockrose (*Cistus ladanifer*) and sparse willow trees (*Salix* sp.) The hydroperiod is variable, but the pond usually fills up with the rains in winter and spring (January-March) and dries up by June-July. Adjacent meadows are usually flooded during the winter and early spring. The other pond (Gravera: 40.8526°N, 3.6538°W, 1006 m a.s.l.) is an old, naturalized gravel pit. It occupies an area of 2,100 m² and has a maximum depth of up to 3 m, with a longer hydroperiod than Laguna (usually from November-December to August). The aquatic vegetation is less abundant, specially dominated by river water-crowfoot (*Ranunculus fluitans*). The two ponds are separated by 700 m in a straight line, with no artificial barriers potentially affecting amphibian dispersal. Other breeding sites in the area include a water trough for cattle, a small stream (La Huesa), two abandoned swimming pools (Pool A and Pool R) and a couple small natural springs (see Results). While we have documented some migration events between these sites and the two main ponds considered in our study, their role in metapopulation dynamics is assumed to be minor because of their smaller size (but see section on dispersal rates below).

2.2. Monitoring program and sampling methods

We monitored the subpopulations of *Pelophylax perezi* in the study area from 2010 to 2019. Details of the surveys are described in Sánchez-Montes *et al.* (2017b), where part of the data of this monitoring program (2010-2015) was analysed. Briefly, we captured all adult frogs found during nocturnal surveys by hand or with dip nets, recorded their sex based on morphological sexually dimorphic characters, and measured their snout-to-vent length (SVL) and their body mass. Individuals captured for the first time were marked with 8 mm passive integrated transponders containing a unique alphanumeric code (PIT tags); for recaptured individuals, codes were read to confirm their identity. We obtained a tissue sample from all captured individuals by clipping the fourth toe of the right hind limb and preserving it in absolute ethanol until DNA extraction.

During the 2018 breeding season we also collected tissue samples for molecular analyses (the fourth toe of the left hind limb) from recently metamorphosed (hereafter metamorphic) individuals in the two main ponds. These individuals were captured soon after metamorphosis, so they were most certainly born in the place of capture during the summer of 2018. These specimens were not individually marked with PIT tags, but we recorded their SVL and body mass. After being processed, all specimens were released back in the place of capture, ensuring they moved normally and were in good condition. All procedures were approved by the Ethics Committee of Consejo Superior de Investigaciones Científicas, Spain (ref.: 710/2018)

and Comunidad de Madrid (ref.: PROEX 040/19), that also extended legal permits for tissue collection for scientific purposes (ref.: 10/069513.9/18).

2.3. Demographic estimates based on CMR

We used MARK software (White & Burnham 1999) to estimate N_a of the two *Pelophylax perezi* subpopulations in every breeding season from 2010 to 2019, the average survival of individuals, and their temporal migration rates based on the capture histories obtained during the monitoring program. Additionally, we analysed separately the 2018 breeding season (when genetic samples were collected) to test for the consistence of estimates obtained with different analytical formulations.

We used the POPAN formulation (Taylor *et al.* 1999), based on the original Jolly-Seber (JS) model (Jolly 1965; Seber 1965) for open populations to analyse the 2018 breeding season. POPAN allows estimation of four main parameters: apparent survival of individuals (ϕ), probability of capture (p), rate of entrance of new individuals in the study area between two sampling occasions ($pent$), and N (in our case corresponding to N_a), estimated as the superpopulation composed of all individuals that had been present at the surveyed area during the study period. We tested different models with potential biological significance, with p and $pent$ considered as either a) time-dependent or b) time-and-sex-dependent and ϕ considered as a) time-dependent, b) sex-dependent, or c) time-and-sex-dependent. For the construction of the matrix of encounter histories for this analysis we considered all sampling occasions in 2018 with a minimum of four captures, because those with fewer captures increase model complexity without a significant contribution to dataset information. In a small number of cases we combined consecutive sampling occasions with few captures into a single occasion. For Laguna we considered a total of six occasions, including 21 individuals (12 males and nine females), and for Gravera a total of 14 occasions, including 60 individuals (42 males and 18 females). Three individuals were captured in the two ponds at different occasions, so they were included in the analyses of both subpopulations.

We used two different formulations of the Robust Design (RD) method: closed RD and open RD to analyse the full data set (2010-2019 breeding seasons). The closed RD (Pollock 1982) is a combination of the Cormack-Jolly-Seber (CJS) live-recapture model and closed capture models. It assumes that the population of study is open to recruitment, mortality and migration between primary occasions (in our case, corresponding to annual breeding seasons), and closed among the secondary occasions nested within a single breeding season, when population size is assumed to remain constant. To avoid strong violations of these assumptions,

we minimized the time intervals between secondary occasions by selecting those closer in time while encompassing the majority of captures. The dataset included capture histories for 271 individuals from Laguna (153 males and 118 females) and 371 individuals from Gravera (245 males and 126 females). The total number of secondary occasions (*i.e.*, sampling sessions) was 41 for Laguna and 68 for Gravera. Whenever possible, we discarded sampling sessions with very few captures to avoid over-parameterization of models, but we maintained at least two secondary occasions per breeding season to meet the requirements of RD. This was the case of year 2012, which was especially dry in Laguna and had almost no breeding activity (see Sánchez-Montes *et al.* 2017b), so we could only include two secondary sampling occasions for this year. We generated a model set by applying constraints (time and/or sex dependence) to annual survival (S) and modelling the probability of temporal migration of individuals out of (γ') or into ($1-\gamma'$) the subpopulation between primary occasions as either a) random, *i.e.* independent of the last probable state of the individual; b) Markovian, *i.e.* dependent of the last probable state of the individual; or c) absent, with temporary immigration/emigration fixed to zero. The probabilities of capture (p) and recapture (c) were always set as equal to each other and modelled as sex- and time- dependent. To account for the possible violation of the assumption of population closure among secondary occasions, we also analysed the same dataset using an open RD multi-state approach (Kendall & Bjorkland 2001; Kendall & Nichols 2002). This formulation, while more complex (more parameters) to implement, is a priori better suited to the biology and population dynamics of *P. perezi*, based on our field observations during the monitoring program. Individuals usually remain in the same pond during consecutive days, but migrate more often (but not exclusively) to other ponds between breeding seasons (Sánchez-Montes *et al.* 2018). We tested models considering ϕ as sex-dependent, and p and $pent$ as time-dependent, based on the top set of models in previous POPAN analyses, and S as sex- or time-dependent. The probability of transition between states *breeder* and *non-breeder* (ψ) was modelled as time-, sex- or state-dependent.

In all analyses in MARK (POPAN and closed and open RD), tested models were ranked based on the Akaike Information Criterion corrected for small sample sizes (AICc) (Burnham & Anderson 2002), and final estimates of parameters along with their 95% confidence intervals (95% CI) were obtained by averaging estimates from the candidate models taking into account their AICc weights (see Supplementary Material). Estimates of N_a were separately obtained for males and females of both subpopulations. To assess the goodness of fit of the saturated models to the data we used the computer application U-CARE (Choquet *et al.* 2009). For both POPAN and RD analyses we tested for the most common causes of departure from CJS model assumptions: “transience” and “trap-dependence” using the tests 3.SR and 2.CT, respectively.

2.4. Genotyping by microsatellites

We genotyped all adult individuals captured in the study area during the 2018 breeding season ($N = 84$): 23 at Laguna (15 males and eight females), 58 at Gravera (42 males and 16 females) and three individuals (one male and two females) that were captured in both ponds (see above). In addition, we genotyped a sample of metamorphic individuals obtained from both subpopulations in 2018 (52 from Laguna and 46 from Gravera). DNA extraction was performed with commercial kits (DNeasy 96 Blood & Tissue Kit, QIAGEN) following the instructions of the manufacturer. All individuals were genotyped using 15 polymorphic microsatellites optimized for the study species (Sánchez-Montes *et al.* 2016), combined in three multiplex reactions. PCRs were performed with Type-it Microsatellite PCR kits (QIAGEN), with PCR cycles as described in Sánchez-Montes *et al.* (2016). Individual genotypes were called using software GeneMapper (Applied Biosystems). For comparison with estimates of genetic diversity in 2018, we reanalysed the genotypes of individuals from Laguna sampled in 2010 and published in Sánchez-Montes *et al.* (2017b).

2.5. Genetic diversity and estimates of N_e and N_b

We analysed the genetic diversity of both subpopulations in 2018, and compared values observed in Laguna in 2010 and 2018 using GenAlEx v6.503 (Peakall & Smouse 2012). We calculated allelic richness, the number of private alleles and observed and expected heterozygosity in each subpopulation and temporal sample. We also calculated Jost's D and Hedrick's G_{ST} as measures of pairwise genetic differentiation between subpopulation samples (Hedrick 2005; Jost *et al.* 2018), using packages "mmod" (Winter 2012) and "adegenet" (Jombart 2008) in R (R Core Team 2017).

We used the sibship frequency (SF) method implemented in software Colony version 2.0.6.1 (Jones & Wang 2010) to reconstruct sibship and parentage and obtain estimates of N_b for the 2018 breeding season in Laguna and Gravera. We used the genotypes of metamorphic individuals as offspring, and those of all males and females from both ponds captured during the 2018 season as candidate parents. We performed analyses assuming a 0.15 probability of parents being in the candidate sample (and tested the effect of this prior on reconstructed pedigrees by comparing results with analyses assuming a 0.5 prior probability) and a prior genotyping error rate of 0.05 for all microsatellites, based on previously estimated error rates in Sánchez-Montes *et al.* (2017a). We allowed for the possibility of multiple matings within a single breeding season in both sexes (polygamy) and ran replicate analyses with the Full-Likelihood method, with "very long" run length and "very high" precision settings. We conducted analyses

with and without a “weak” sibship size prior = 1 for both paternal and maternal families. To estimate N_e in each subpopulation we used the genotypes of all adults as offspring, with no parental candidates, under the same settings used for N_b analyses. Based on the inferred pedigrees for 2018 and the pedigrees for 2010 obtained from Sánchez-Montes *et al.* (2017b), we estimated the breeding success of males and females in each subpopulation as the proportion of inferred sires and dams (respectively) compared to the total estimated adult population of each sex (N_a). Additionally, we estimated the average number of mating pairs for individuals of each sex from the pedigrees, and the percentage of monogamous and polygamous males and females in each subpopulation.

2.6. Migration rates

We summarized the frequency and direction of dispersal events of marked individuals in the study area based on their capture histories. Additionally, we calculated accumulated distances of dispersing individuals as a minimum estimate of their dispersal potential.

To infer whether observed displacements resulted in gene flow, we estimated migration rates per generation between the two subpopulations based on individual adult genotypes from the 2018 breeding season with software BayesAss (Wilson & Rannala 2003). BayesAss assumes that migration rates can be asymmetric but are constant over short periods of time, and that migration rates are small (Faubet *et al.* 2007). Ten replicate analyses with different random seeds were run for 10,000,000 iterations, discarding the first 2,000,000 iterations as burn-in; the MCMC mixing parameters of allele frequencies (a), inbreeding coefficients (f) and migration rates (m) were 0.45, 0.40 and 0.30, respectively. These values provided acceptance rates between 20% and 60% in MCMC chains, which are optimal according to the software manual. We assessed convergence of migration rate estimates across replicate runs by checking logfiles with Tracer software (Rambaut *et al.* 2018). The probability of ancestry for each individual was divided into three categories: non-migrant (resident), first-generation migrant, and second-generation migrant.

As a complementary, indirect approach, we tested for differences in seven pairwise relatedness indices calculated with software Coancestry (Wang, 2011) between individuals from different ponds and sexes, using non-parametric Wilcoxon signed-rank tests. Our expectations are that 1) the more philopatric sex will show significantly higher values of relatedness; and 2) individuals from the pond receiving more migrants will show significantly lower values of relatedness.

3. RESULTS

3.1. Monitoring program and CMR data

A total of 647 frogs (246 females and 401 males) were marked during the study. Of these, 389 frogs (141 females and 248 males) were captured more than once (60.1% global recapture rate). Recapture rates (*i.e.*, the proportion of individuals captured more than once during the ten-year monitoring) were 54.6% in Laguna and 63.1% in Gravera. The number of captures and recaptures in Gravera increased every breeding season, but in Laguna there were few new captures and recaptures from 2011 to 2018, with a slight increase in 2019 (Fig. 1). The sex-ratio in our sample was male-biased in Gravera (1.9:1) but closer to 1:1 in Laguna (1.3:1).

3.2. Demographic estimates based on CMR

We did not detect departures from Cormack-Jolly-Seber (CJS) model assumptions among sampling sessions in Laguna or Gravera, neither in the POPAN dataset nor among secondary occasions in the open and closed Robust Design (RD) formulations, with the exception of males from Gravera showing slight “trap-dependence” effects in 2011, 2017 and 2018 in RD analyses (Table S1). Estimates of N_a for both sexes were consistent between POPAN (Table S2) and the open (Table S3) and closed (Table S4) RD formulations for the 2018 breeding season. Moreover, the open and closed RD formulations provided similar results for all years. Thus, hereafter we report estimates obtained with the open RD formulation, because relaxation of the closure assumption within each breeding season accommodates better the biology and population dynamics of the species, despite the cost of increased model complexity.

Based on open RD results, the best-ranked models in each subpopulation were those where the apparent survival of individuals (ϕ) was set as sex-dependent, and the probability of transition between states *breeder* and *non-breeder* (ψ) as sex or state-dependent (Table S3). Figure 2 summarizes estimates of N_a across breeding seasons for Laguna and Gravera. The precision of estimates increased with accumulated sampling effort, especially since 2011 in Gravera and 2013 in Laguna. An exception was the 2019 breeding season in Laguna, when numerous small-sized, unmarked individuals (mostly males) entered the population. At the beginning of our study, Laguna hosted a larger population, but following the dry breeding season of 2012 the population sharply declined, with an apparent recovery in 2019 (but see the uninformative 95% CI of males, Fig. 2). The population at Gravera followed a similar pattern of decline after 2012, but recovered sooner, in the breeding season of 2017 (Fig. 2).

3.3. Genetic diversity and estimates of N_e and N_b

All samples were genotyped successfully, with 0.26% of missing data in the final genotype matrix. A subset (19.23%) of samples were genotyped twice, with consistent results in all cases. Table 1 summarizes genetic diversity indices for both populations and for the two temporal samples of Laguna. Observed heterozygosity was similar in Laguna in 2010 and 2018 and in Gravera in 2018. However, we detected a reduction in allelic richness and in the mean number of private alleles from 2010 to the present in Laguna, with a general loss of diversity in most loci (Fig. S1). Both estimates of genetic differentiation, D and G_{ST} , showed higher differentiation between Laguna and Gravera (more than double, regardless of the sampling year in Laguna) than between temporal samples in Laguna (Table 2).

Estimates of N_b and N_e for the 2018 breeding season are summarized in Table 3. We observed a sharp decrease in N_b (from 69 to 21) and N_e (from 49 to 25) in Laguna in the 2010 – 2018 period, and a parallel increase in the N_b/N_a ratio (from 0.5 to 0.95). Estimates of N_b and N_e in Gravera were higher than in Laguna in the 2018 season, but lower than in the 2010 season. The N_b/N_a ratio was smaller in Gravera compared to Laguna in both time periods (Table 3).

Inferred pedigrees were concordant across the different runs and prior settings. Most inferred parents (81%) of the 2018 metamorphic individuals were among the genotyped samples of candidate fathers and mothers (Table S5). Breeding success varied across breeding sites and sexes, showing maximum values in Laguna 2018, both for males and females, and minimum value for males in Gravera (Table 4). According to inferred parentage relationships, half or more of the successful breeders in Laguna mated with only one partner (54% and 58% of males and females in 2010 and 78% and 50% of males and females in 2018, respectively). The percentages in Gravera were lower, with 47% and 40% of successful male and female breeders mating with a single partner. The average number of mating partners per successful breeding individual was similar for both sexes and subpopulations (1.4-1.7), except for females in Laguna 2018, which showed higher values (2.1, Table 4).

3.4. Migration rates

Figure 3 summarizes the frequencies and directions of dispersal events recorded during our ten-year study. A total of 78 adult individuals (46 males, 32 females) were captured in multiple locations (20.1% of frogs captured more than once), traveling accumulated distances between 160 and 2,200 m (Fig. S2). The locations of capture of all individuals with recorded displacements are shown in Table S6. Most displacements involved distances < 1,000 m. We did not find strong differences in the dispersal capacity of males vs. females, considering the

number of dispersal events and the minimum accumulated distance travelled per individual (Table S6).

One of the three genotyped migrants (captured both in Laguna and in Gravera in 2018) was inferred to be mother of metamorphic individuals captured at both breeding sites. In addition, we detected cryptic migration events in three additional individuals that were only captured in Gravera, with two of them inferred to have sired offspring in both ponds, and the other one breeding in Laguna (Table S5).

Results of ten replicate analyses in BayesAss were consistent, with likelihood scores of -3965.59 to -3968.32, so we report estimates for the run with the best likelihood. In Laguna, 96% of individuals were inferred to be migrants from Gravera, with only one sampled individual being assigned to the resident class. In Gravera, 53% of individuals were classified as residents, and 47% as migrants or descendants of migrants from Laguna (Fig. 4). Migration between the two ponds is asymmetrical, with a higher migration rate from Gravera to Laguna (29.9%, 95% highest posterior density interval -HPDI: 25.4 – 33.3%) than in the opposite direction (16.2%, 95% HPDI: 11.3 – 21.1%).

The seven estimators of relatedness showed consistent differences between Laguna and Gravera, with individuals from Laguna presenting lower average relatedness than individuals from Gravera (Table S7). All estimators showed statistically significant differences when comparing all individuals from Laguna 2010 and 2018 pooled together vs Gravera 2018, but some differences were not significant when comparing only individuals captured in 2018. We did not observe differences in average relatedness between males and females (Table S7).

4. DISCUSSION

From a conservation perspective, it is important to characterize demographic dynamics of populations based on census estimates through time, because this can provide information about their viability in the long-term (Green 1997). Nevertheless, capture-mark-recapture (CMR) methods require exhaustive population monitoring, and intensive field work is essential to obtain reliable estimates (Donnelly & Guyer 1994). In our 10-year monitoring program, we obtained high global recapture rates (60.1%), especially in Gravera (Fig. 1). Consequently, we produced robust estimates of N_a , with narrow 95% CIs for both sexes across years (Fig. 2 and Table S3). Furthermore, results of demographic estimates using different formulations and models in CMR analyses were largely concordant, so they can be used to reliably infer demographic trends in each subpopulation.

Based on our results, both subpopulations have experienced different dynamics in the last decade. At the beginning of our monitoring program, Laguna hosted a larger frog population than Gravera (almost x3, Table S3). However, after the dry season of 2012 (Sánchez-Montes *et al.* 2017b), there was a sharp decline in N_a at Laguna, where the population decreased down to a minimum of 14 individuals in 2017. The subpopulation at Gravera also experienced a decline after the 2012 drought, but it was not so severe, and the population recovered faster (during the 2017 breeding season, Fig. 2). A possible reason for the different response of both subpopulations to the drought is the longer hydroperiod of Gravera (see Methods). A short hydroperiod imposes serious constraints on the long development of *Pelophylax perezii* tadpoles (Richter-Boix *et al.* 2006). In fact, we observed little or no metamorphic recruitment in Laguna in most years from 2012 to 2017, which contrasted with abundant yearly recruitment in Gravera (unpublished data). The difference in hydroperiod can thus confer Gravera a key role as a more stable subpopulation, less sensitive to stochastic perturbations like dry years.

The demographic trend in Laguna since 2012 reflects a population bottleneck (Fig. 2). The consequences of this bottleneck included a substantial genetic impoverishment across the two time periods analysed, with both allelic richness and the number of private alleles decreasing sharply from 2010 to 2018 (Table 1). Patterns of allele loss were shared across markers (Fig. S1), whereas heterozygosity estimates were similar across temporal samples and subpopulations. This is consistent with theoretical expectations, with allelic richness being more sensitive to severe reductions of population size due to the faster loss of rare alleles, which contribute little to average heterozygosity (Leberg 2002; England *et al.* 2003). Levels of genetic diversity at Gravera were similar to those found in Laguna at 2010, albeit with a lower number of private alleles in Gravera. While we have no information about genetic diversity in Gravera in the past, based on the more stable demographic profile of the subpopulation we would expect similar levels of allelic richness through time. At any rate, the observed loss of genetic diversity at Laguna could eventually result in inbreeding depression, in the absence of migration between the two subpopulations.

Genetic consequences of the bottleneck at Laguna are also apparent in N_e estimates, which declined from 49 in 2010 to 25 in 2018 (Table 3). Again, the N_e estimate at Gravera (38) was similar to N_e in Laguna in 2010. These estimates are well below threshold values proposed to avoid inbreeding depression ($N_e > 50-100$) and maintain the evolutionary potential of the population ($N_e > 1000$), so both populations can be considered to be vulnerable to genetic drift (Phillipsen *et al.*, 2011; Lourenço *et al.*, 2018). Regarding N_b estimates, we also observed a severe reduction in Laguna between 2010 (69) and 2018 (21); however, the N_b/N_a ratio was higher in 2018 than in 2010 (Table 3). The high value estimated in 2018 (0.95) may reflect

plasticity or genetic compensation mechanisms, which have been previously described in small sized populations facing the loss of genetic diversity (Jehle *et al.* 2005; Palstra & Ruzzante 2008; Beebee 2009). In large populations, the operational sex-ratio (OSR), referring to the proportion of both sexes coinciding at the same time in the breeding area, can be strongly biased towards males, resulting in male-male competence and high variance in individual breeding success. Instead, in small populations the OSR can be less biased, which helps maximize the individual genetic contribution to the next generation and increase the N_b/N_a ratio (Beebee 2009; Ficetola *et al.* 2010). In our study site, the high N_b/N_a ratio may be a consequence of increased average individual breeding success in both sexes (Table 4), which was confirmed by reconstructed pedigrees in Laguna in 2018 (Table S5). This contrasts with estimates at Laguna in 2010, when breeding success was lower in both males and females and, concordantly, the N_b/N_a ratio was lower (Table 3). The average number of partners per successful breeding individual in Laguna in 2010 was 1.6 in both sexes (Sánchez-Montes *et al.* 2017b). In 2018, both populations showed similar values, between 1.4 and 1.7 in both sexes, but slightly higher (2.1) in females in Laguna (Table 4). Thus, the high individual breeding success of Laguna does not seem to be associated with a high increase in the mean number of mating partners per individual (at least in the case of males), suggesting that variation in the N_b/N_a ratio is not driven by polygamy rates in this system.

In a metapopulation context, connectivity between subpopulations may buffer the effects of local decreases in genetic diversity through migration. Both our field observations and molecular analyses provide evidence of connectivity between the two subpopulations, with no signs of sex-biased dispersal according to relatedness estimates. A total of 18 individuals (eight males, eight females and one juvenile, Table S6) have been observed in the two ponds at different times during the monitoring program, sometimes during the same breeding season, highlighting the high dispersal capacity of the species (Sánchez-Montes *et al.* 2018 and unpublished data). Based on inferred parentage relationships, we also recorded several “cryptic” dispersal events in the 2018 breeding season, in addition to the three individuals that were captured in both ponds, one of which successfully reproduced in the two sites (Table S5). This underlines the importance of genetic analyses to complement CMR monitoring programs and provide robust inferences on functional connectivity across subpopulations.

Estimates of migration rates with BayesAss revealed asymmetries in the direction of dispersal between the two subpopulations, with rates from Gravera to Laguna (29%) almost doubling those estimated for the opposite direction. Considering that BayesAss can detect a maximum migration rate of 33.3% (Faubet *et al.* 2007), our estimate of the former rate can be an underestimate, since the 95% highest posterior density interval included that maximum

value. This asymmetry in migration rates is consistent with the idea that dispersal is density-dependent in this system, since frog density is higher in Gravera. Density effects could partly explain the low N_b/N_a ratio in Gravera, where competition for mates and breeding sites may be stronger than in Laguna. Remarkably, this preferred direction of movement runs counter to the topography of the study area, with frogs migrating from Gravera to Laguna overcoming a 60 m difference in altitude. At any rate, this high inferred migration rate is consistent with field data and the observed high dispersal capacity of the species based on the relatively great number of long distance (700 m) dispersal events (Sánchez-Montes *et al.* 2018), and compared to other co-distributed amphibian species like *Pleurodeles waltl* and *Pelobates cultripes* (Gutiérrez-Rodríguez *et al.* 2017). In addition, low estimates of genetic differentiation between subpopulations are consistent with inferred levels of gene flow (Table 2). The asymmetry of migration was further supported by the lower average relatedness observed among individuals from Laguna.

Inspection of individual ancestry probabilities further revealed that approximately 50% of individuals in Gravera were residents and 50% were 1st or 2nd generation migrants from Laguna, whereas most individuals in Laguna were inferred to be migrants from Gravera (Fig. 4). Considering that the life span of *Pelophylax perezi* is inferred to be around 6-8 years (Sánchez-Montes *et al.* 2017b), these results are consistent with a possible rescue effect, with individuals from Gravera recolonizing Laguna after the 2012 drought. It should be kept in mind that migration rates can change through time, and thus values in the past may have been closer to symmetry (or asymmetric in the opposite direction, as observed for *Pleurodeles waltl* in the study area, unpublished data). Additional evidence for a rescue effect comes from our field observations in 2019. That year, a large number of small-sized frogs were captured at Laguna, where no juvenile recruitment had been observed in the 2012-2017 period. This resulted in poor estimates of N_a , especially in males (Fig. 2 and Table S3). Pedigrees from Laguna in 2018 showed that most metamorphs were descendants from adults captured in Laguna, which in turn have been classified as migrants from Gravera (Fig. 4 and Table S5). This supports the idea that present recruitment in Laguna is due to a rescue effect mediated by individuals migrating from Gravera.

Overall, our integrative monitoring approach provided valuable insights about population dynamics of a pond-breeding amphibian over an extended period of time. The combination of field-based CMR estimates and multilocus genotypes allowed characterization of population trends and migration rates, highlighting the role of the pond with a longer hydroperiod (Gravera) as a metapopulation source, potentially capable of rescuing the shorter-hydroperiod, bottlenecked population from genetic loss through migration. The migration rate

of the species, higher than in other syntopic amphibians, is probably one of the reasons they thrive where other species decline, because local declines and extinctions can be compensated by recolonization events. Our results have important conservation implications, because while Laguna is legally protected, Gravera, which has a key role in the persistence of the metapopulation, is not under any conservation category. Management plans for the protection of wetlands and their associated communities will benefit from more integrative assessments of population demography and connectivity, ensuring that subpopulations with a stronger leverage on metapopulation resilience are effectively protected and managed.

ACKNOWLEDGMENTS

All procedures were approved by the Ethics Committee of Consejo Superior de Investigaciones Científicas, Spain (ref.: 710/2018) and Comunidad de Madrid (ref.: PROEX 040/19), which also extended legal permits for tissue collection for scientific purposes (ref.: 10/069513.9/18). This study was funded by FEDER / Ministerio de Ciencia, Innovación y Universidades –Agencia Estatal de Investigación, Spain, grant CGL2017-83131-P. We thank L. Arregui, C. Caballero, M. Durán, I. Fernández de Larrea, E. Iranzo, D. Jiménez, H. Martínez, M. Peñalver, R. Plaza, S. Sánchez, G. Ugarte for help during field work.

REFERENCES

- Allentoft ME, Brien JO (2010). Global amphibian declines, loss of genetic diversity and fitness: A Review. *Diversity* **2**, 47–71.
- Beebee TJC (2009). A comparison of single-sample effective size estimators using empirical toad (*Bufo calamita*) population data: genetic compensation and population size-genetic diversity correlations. *Molecular Ecology* **18**(23), 4790–4797.
- Betini GS, Fitzpatrick MJ, Norris DR (2015). Experimental evidence for the effect of habitat loss on the dynamics of migratory networks. *Ecology Letters* **18**, 526–534.
- Bode M, Burrage K, Possingham HP (2008). Using complex network metrics to predict the persistence of metapopulations with asymmetric connectivity patterns. *Ecological modelling* **214**, 201–209.
- Buckley LB, Jetz W (2007). Environmental and historical constraints on global patterns of amphibian richness. *Proc. R. Soc. B.* **274**, 1167–1173.
- Burnham KP, Anderson DR (2002). Model selection and multimodel inference: a practical information-theoretic approach. 2nd Ed. Springer-Verlag, New York, USA

Cayuela H (2016). Demographic responses to weather fluctuations are context dependent in a long-lived amphibian. *Global Change Biology* **22**, 2676–2687.

Charlesworth B (2009). Effective population size and patterns of molecular evolution and variation. *Nature Reviews* **10**, 195–205.

Choquet R, Lebreton J, Gimenez O, Reboulet AM, Pradel R (2009). U-CARE: Utilities for performing goodness of fit tests and manipulating CAPture–REcapture data. *Ecography* **32**, 1071–1074.

Dias PC (1996). Sources and sinks in population biology. *Trends in Ecology & Evolution* **11**, 326–329.

Donnelly MA, Guyer C (1994). Patterns of reproduction and habitat use in an assemblage of Neotropical hyliid frogs. *Oecologia* **98**, 291–302.

England PR *et al.* (2003). Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. *Conservation Genetics* **4**, 595–604.

Fahrig L, Merriam G (1985). Habitat patch connectivity and population survival. *Ecology* **66**, 1762–1768.

Faubet P, Waples R, Fourier UJ (2007). Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. *Molecular Ecology* **16**, 1149–1166.

Ferchaud A *et al.* (2016). Making sense of the relationships between N_e , N_b and N_c towards defining conservation thresholds in Atlantic salmon (*Salmo salar*). *Heredity* **11**, 268–278.

Ficetola GF, Wang J, Garner TWJ (2010). Polygyny, census and effective population size in the threatened frog. *Animal Conservation* **13**, 1–8.

Fisher RA (1930). The genetical theory of natural selection. Clarendon Press, Oxford

Frankham R (1995). Effective population size/adult population size ratios in wildlife: a review. *Genetic Resources* **66**, 95–107.

Furrer RD, Pasinelli G (2015). Empirical evidence for source – sink populations: a review on occurrence, assessments and implications. *Biological Reviews* **91**, 782–795.

Gonzalez A *et al.* (1998). Metapopulation dynamics, abundance and distribution in a microecosystem. *Science* **281**, 2045–2047.

Green DM (1997). Perspectives on amphibian population declines: Defining the problem and searching for answers. *Herpetological Conservation* **1**, 291–308.

Griffiths RA, Sewell D, McCrea RS (2010). Dynamics of a declining amphibian metapopulation: Survival, dispersal and the impact of climate. *Biological Conservation* **143**, 485–491.

Gutiérrez-Rodríguez J, Sánchez-Montes G, Martínez-Solano I (2017). Effective to census population size ratios in two Near Threatened Mediterranean amphibians: *Pleurodeles waltl* and *Pelobates cultripes*. *Conservation Genetics* **18**, 1201–1211.

Habel JC, Zachos FE (2013). Past population history versus recent population decline – founder effects in island species and their genetic signatures. *Journal of Biogeography* **40**, 206–207.

Hanski I, Schulz T, Wong SC, Ahola V, Ruokolainen A, Ojanen SP (2017). Ecological and genetic basis of metapopulation persistence of the Glanville fritillary butterfly in fragmented landscapes. *Nature Communications* **8**, 1–11.

Hare MP, Nunney L, Schwartz MK *et al.* (2011). Understanding and estimating effective population size for practical application in marine species management. *Conservation Biology* **25**(3), 438–449.

Hedrick PW (2005). A standardized genetic differentiation measure. *Evolution* **59**, 1633–1638.

Hufbauer RA, Szűcs M, Kasyon E *et al.* (2015). Three types of rescue can avert extinction in a changing environment. *Proceedings of the National Academy of Sciences* **112**, 10557–10562.

Jangjoo M, Matter SF, Roland J, Keyghobadi N (2016). Connectivity rescues genetic diversity after a demographic bottleneck in a butterfly population network. *Proceedings of the National Academy of Sciences* **113**, 10914–10919.

Jehle R, Wilson GA, Arntzen JW, Burke T (2005). Contemporary gene flow and the spatio-temporal genetic structure of subdivided newt populations (*Triturus cristatus*, *T. marmoratus*). *Journal of Evolutionary Biology* **18**, 619–628.

Jolly GM (1965). Explicit estimates from capture-recapture data with both death and immigration-stochastic model. *Biometrika* **52**, 225–248.

Jombart T (2008). Adegnet: a R package for the multivariate analysis of genetic markers. *Bioinformatics Applications Note* **24**, 1403–1405.

Jones, O. R., & Wang, J. (2010). COLONY: A program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, **10**, 551–555.

Jost L, Archer F, Flanagan S, Gaggiotti O, Hoban S, Latch E (2018). Differentiation measures for conservation genetics. *Evolutionary Applications* **11**, 1139–1148.

Kendall WL, Bjorkland R (2001). Using open robust design models to estimate temporary emigration from capture-recapture data. *Biometrics* **57**, 1113–1122.

Kendall W L, Nichols JD (2002). Estimating state-transition probabilities for unobservable states using capture – recapture/resighting data. *Ecology* **83**, 3276–3284.

Lacy RC (1987). Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology* **1**, 143–158.

Lande R, Shannon S (1996). The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* **50**, 434–437.

Leberg PL (2002). Estimating allelic richness: Effects of sample size and bottlenecks. *Molecular Ecology* **11**, 2445–2449.

Lee AM, Engen S, Sæther B (2011). The influence of persistent individual differences and age at maturity on effective population size. *Proc. R. Soc. B.*, **278**, 3303–3312.

Lourenço A, Antunes B, Wang IJ, Velo-Antón G (2018). Fine-scale genetic structure in a salamander with two reproductive modes: Does reproductive mode affect dispersal? *Evolutionary Ecology* **32**, 699–732.

Levins R (1969). Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America* **15**, 237-240.

McEachern MB, Van Vuren DH, Floyd CH, May B, Eadie JM (2011). Bottlenecks and rescue effects in a fluctuating population of golden-mantled ground squirrels (*Spermophilus lateralis*). *Conservation Genetics* **12**, 285–296.

Nunney L, Elam DR (1994). Estimating the effective population size of conserved populations. *Conservation Biology* **8**, 175–184.

Palstra FP, Fraser DJ (2012). Effective/census population size ratio estimation: a compendium and appraisal. *Ecology and Evolution* **2**, 2357–2365.

Palstra F, Ruzzante DE (2008). Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Molecular Ecology* **17**, 3428–3447.

Peakall R, Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research — an update. *Bioinformatics Applications Note* **28**, 2537–2539.

Phillipsen IC, Funk WC, Hoffman EA, Monsen KJ, Blouin MS (2011). Comparative analyses of effective population size within and among species: ranid frogs as a case study. *Evolution* **65**, 1–19.

Pollock KH (1982). A capture-recapture design robust to unequal probability of capture. *Journal of Wildlife Management* **46**, 757–760.

R Core Team (2017). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**, 901–904.

Richter-Boix A, Llorente GA, Montori A (2006). Breeding phenology of an amphibian community in a Mediterranean area. *Amphibia-Reptilia* **27**, 544–549.

Sánchez-Montes, G., Ariño, A. H., Vizmanos, J. L., Wang, J., & Martínez-Solano, I. (2017a). Effects of sample size and full sibs on genetic diversity characterization: A case study of three syntopic Iberian pond-breeding amphibians. *Journal of Heredity*, **108**, 535–543.

Sánchez-Montes G, Wang J, Ariño AH, Vizmanos JL, Martínez-Solano I (2017b). Reliable effective number of breeders/adult census size ratios in seasonal-breeding species: Opportunity for integrative demographic inferences based on capture–mark–recapture data and multilocus genotypes. *Ecology and Evolution* **7**, 10301–10314.

Sánchez-Montes G, Wang J, Ariño AH, Martínez-Solano I. (2018). Mountains as barriers to gene flow in amphibians: quantifying the differential effect of a major mountain ridge on the genetic structure of four sympatric amphibian species with different life history traits. *Journal of Biogeography* **45**, 318–331.

Sánchez-Montes G, Martínez-Solano I (2011). Population size, habitat use and movement patterns during the breeding season in a population of Perez's frog (*Pelophylax perezi*) in central Spain. *Basic and Applied Herpetology* **25**, 81–96.

Sánchez-Montes G, Recuero E, Gutiérrez-Rodríguez J, Gomez-Mestre I, Martínez-Solano I (2016). Species assignment in the *Pelophylax ridibundus* x *P. perezi* hybridogenetic complex based on 16 newly characterised microsatellite markers. *Herpetological Journal* **26**, 99–108.

Seber GAF (1965). A note on the multiple-recapture census. *Biometrika* **52**, 249–260.

Shtilermam E, Stone L (2015). The effects of connectivity on metapopulation persistence: network symmetry and degree correlations. *Proc. R. Soc. B*, **282**, 1–8.

Sonsthagen SA, Wilson RE, Underwood JG (2017). Genetic implications of bottleneck effects of differing severities on genetic diversity in naturally recovering populations: An example from Hawaiian coot and Hawaiian gallinule. *Ecology and Evolution* **7**, 9925–9934.

Taylor AD, Harrison S (1997). Empirical evidence for metapopulation dynamics. In: Hanski I, Gilpin ME, ed. *Metapopulation Biology: ecology, genetics, and evolution*, Academic Press, San Diego, pp. 27–42.

Taylor P, Arnason AN, Schwarz CJ (1999). Using POPAN-5 to analyse banding data. *Bird Study* **46**, 157–168.

Thomas CD, Kunin WE (1999). The spatial structure of populations. *Journal of Animal Ecology* **68**, 647–657.

Wang, J. 2011. COANCESTRY: A program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources*, **11**, 141–145.

Waples RS, Luikart G, Faulkner JR, Tallmon DA (2013). Simple life-history traits explain key effective population size ratios across diverse taxa. *Proc. R. Soc. B.*, **280**: 20131339.

Waples RS, Antao T, Luikart G (2014). Effects of overlapping generations on linkage disequilibrium estimates of effective population size. *Genetics* **197**, 769–780.

White G C, Burnham KP (1999). Program MARK: survival estimation from populations of marked animals. *Bird Study* **46**, 120–139.

Wilfried G, Bürger R (1992). Survival of small populations under demographic stochasticity. *Theoretical Population Biology* **41**, 44–71.

Wilson GA, Rannala B (2003). Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163**, 1177–1191

Winter DJ (2012). MMOD: an R library for the calculation of population differentiation statistics. *Molecular Ecology Resources* **12**, 1158–1160.

Wright S (1931). Evolution in Mendelian Populations. *Genetics* **16**.

Xenikoudakis G, Ersmark E, Tison JL, Waits L, Kindberg J, Swenson JE, Dalén L (2015). Consequences of a demographic bottleneck on genetic structure and variation in the Scandinavian brown bear. *Molecular Ecology* **24**, 3441–3454.

TABLES

Table 1 Genetic diversity indices estimated from multilocus genotypes for Laguna (2010 and 2018) and Gravera (2018). N = mean sample size; AR = mean allelic richness; PA = mean number of private alleles; H_0 = observed heterozygosity; H_E = expected heterozygosity.

Subpopulation	Year	N	AR	PA	H_0	H_E	Table 2 Pairwis
Laguna	2010	93.2	10.87	1.67	0.76	0.75	
Laguna	2018	23.0	8.00	0.20	0.76	0.74	
Gravera	2018	57.5	10.07	0.73	0.73	0.74	

e estimates of genetic distances (Jost's D , upper diagonal and Hedrick's G_{ST} , lower diagonal) between subpopulations and temporal samples.

	Laguna 2010	Laguna 2018	Gravera 2018
Laguna 2010	-	0.023	0.054
Laguna 2018	0.031	-	0.063
Gravera 2018	0.071	0.082	-

Table 3 Estimates of N_a (with 95% confidence intervals, 95% CI), number of inferred (Inf.) parents, estimates of N_b , N_e (with 95% CI) and the N_b/N_a ratio for the subpopulations (Subpop.) Laguna (2010 and 2018) and Gravera (2018).

Subpop.	Year	N_a			Inf.		N_b	N_e	N_b/N_a ratio
		Males	Females	Total	sires	dams			
Laguna	2010	69 (30 - 108)	68 (4 - 133)	137	37	38	69 (49 - 98)	49 (33 - 74)	0.50
Laguna	2018	14 (12 - 16)	8 (7 - 9)	22	18	12	21 (12 - 39)	25 (15 - 46)	0.95
Gravera	2018	57 (49 - 65)	24 (21 - 28)	81	19	20	41 (26 - 66)	38 (25 - 61)	0.41

Table 4 Number of male and female breeders in the inferred pedigrees of each subpopulation (see Table S5), number of breeders with one or more inferred mating partners and mean

number of inferred mating partners per breeder. The average breeding success of both sexes in each subpopulation is calculated as the proportion of inferred sires and dams (breeders) with respect to the adult population (N_a) as estimated in open RD analyses.

	Laguna 2010		Laguna 2018		Gravera 2018	
	Males	Females	Males	Females	Males	Females
Number of Breeders	37	38	18	12	19	20
Breeders with 1 mating partner	20	22	14	6	9	8
Breeders with >1 mating partner	17	16	4	6	10	12
Mean number of partners per breeder	1.6	1.6	1.4	2.1	1.7	1.7
N_a estimate	69	68	14	8	57	24
Average breeding success	0.54	0.56	1	1	0.33	0.83

FIGURES

Figure 1 Accumulated number of individuals captured once and more than once (i.e., recaptures) for males and females in each breeding season from 2010 to 2019 in Laguna and Gravera

Figure 2 Annual N_a estimates (with 95% CI) by sex obtained in open RD analyses for both subpopulations.

Figure 3 Map of the study area showing the direction and frequency of dispersal events recorded during the ten-year CMR monitoring program (colour codes as in previous figures). The locations of Laguna (L), Gravera (G) and other minor sites where individuals of *P. perezii* were captured are indicated. Cryptic displacements (*i.e.*, those detected based on inferred pedigrees) are not represented in the figure (see Table S6).

Figure 4 Probability of classification as resident or migrant (first-generation migrant and second-generation migrant) for each individual genotyped in Laguna and Gravera.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/1749-4877.12449](https://doi.org/10.1111/1749-4877.12449).