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A genetically-informed Population Viability Analysis reveals conservation priorities for an isolated population of *Hyla arborea*

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Abstract. Population Viability Analysis (PVA) is a commonly used tool to predict the fate of endangered populations. However, although amphibians are the most endangered group of vertebrates, PVAs have so far been underrepresented in their conservation management. In the last decades, the European tree frog (*Hyla arborea*) has experienced drastic declines mainly caused by habitat fragmentation and loss of suitable breeding sites. In the present study, we used the PVA software VORTEX to predict the viability of a *H. arborea* population of about 70 adults inhabiting an isolated pond in the region of Hannover (Germany), by combining life history data with genotypic information derived from eight polymorphic microsatellite markers. Our PVA revealed a high probability of extinction within the next 50 years, with juvenile survival being a crucial demographic parameter for population persistence. Simulated immigration through metapopulation processes or population supplementation prevented genetic erosion, and markedly increased the probability of population survival. Future management interventions should consider pond management to enhance survival at early stages, and the creation of migration corridors to facilitate connectivity with adjacent demes and/or the translocation of individuals. To our knowledge, this is one of the first studies that applies a genetically-informed PVA to the management of endangered anuran amphibians.

Key words. Population viability analysis, *Hyla*, amphibian population decline, genetic diversity, habitat fragmentation.

Introduction

Amphibians are the most endangered group of vertebrates, and their rapid decline in the last decades is primarily attributable to habitat loss and modification (Stuart et al. 2004, Cushman 2006). Habitat fragmentation can rapidly lead to the isolation of populations, which ultimately inhibits genetic exchange and thereby contributes to a loss of overall genetic diversity (Frankham et al. 2010). For conservation management, a central question is whether species and populations are capable of persisting in isolated habitat patches or need regional connectivity for long-term survival (Marsh 2008).

Population Viability Analysis (PVA) is an important demographic tool to quantify the extinction risk of populations. Taking life-history and environmental data into account, PVA uses computer models to simulate population trajectories, and, perhaps most importantly, creates a basis for evaluating the influences of factors contributing to vulnerability and decline. A suite of software packages is available for PVAs (e.g., VORTEX [Lacy & Pollak 2014], RAMAS [Akçakaya & Sjögren-Gulve 2000], and ALEX [Possingham & Davies 1995]). However, despite a wide use for higher vertebrates, PVAs are as yet underrepresented in amphibian studies (but see Hels & Nachman 2002, Stevens & Baguette 2008, Greenwald 2010, Arntzen 2015), a fact that is likely attributable to their high fecundity and a general lack of individual life tables.

Genetic factors are important to determine the conservation status of populations, and it is widely accepted that a joint consideration of demographic, ecological and genetic processes should assess the threats posed by population isolation (Greenwald 2010, Olsen et al. 2014, Pierson et al. 2015). For example, the amount of genetic erosion in isolated demes is determined by the effective population size, which can be quantified with genetic means using markers such as microsatellites (Jehle & Arntzen 2002, Selkoe & Toonen 2006). Genetic erosion is assumed to negatively influence population-wide levels of fitness through decreased heterozygosities (e.g., Luquet et al. 2011), with low effective population sizes also increasing the likelihood of inbreeding (Edenhamn et al. 2000, Rowe & Beebee 2003, Spielman et al. 2004). However, the inclusion of genetic processes into PVAs is so far poorly represented (Reed et al. 2002, Pierson et al. 2015).

The European tree frog (*Hyla arborea*) is a typical temperate-climate amphibian species affected by human habitat alteration. Modification and destruction of breeding sites have caused sharp declines in the last decades. Across a range of landscapes inhabited by *H. arborea*, a clear link between increased habitat fragmentation and genetic deterioration is pertinent (Arens et al. 2000, Edenhamn et al. 2000, Andersen et al. 2004, Broquet et al. 2010, Krug & Proehl 2013). The vulnerability of *H. arborea* especially in its northern and western ranges is additionally attributed to postglacial population expansions from glacial refugia owing to decreasing genetic diversity along the expansion route (Dufresnes et al. 2013). The present study deals with an isolated population of *H. arborea* in the region of Hannover (Germany). Within a cluster of fragmented demes, this population was established by translocation in the mid-1980s, with genetic evidence suggesting a propagule ~20 km away (Krug & Proehl 2013). The purpose of the present study is to (i) predict the viability for the isolated population by incorporating demographic as well as genetic data into a PVA, (ii) identify the life history parameters with the greatest impact on population persistence, and (iii) examine the possibility of immigration (natural connectivity with, or supplementation from, the source population) to enhance population survival. In addition to the genetically-informed PVA we also calculated effective population sizes (N_e) by comparing three complementary approaches.

Materials and methods

Fieldwork was undertaken in 2005 and 2008 as part of a wider study (Krug & Proehl 2013 and unpublished data). The isolated study population ("Benther Berg") lies west of Hannover (Germany) and stems from an unapproved introduction in 1984 or 1985, with the propagule population ~20 km east of it (Krug & Proehl 2013). It consists of two ponds at 30 m distance connected through irregularly flooded reed beds. The distance to the next known occurrence of *H. arborea* is ~6 km. Although the population size is small, it remained relatively stable within the last 20 years (Arbeitsgemeinschaft Biotop- und Artenschutz GbR, ABIA, unpubl. data).

Genotyping and genetic measurements of population size

DNA samples were obtained by buccal swabs collected during the spring breeding season. A total of 28 samples were obtained (2005: 17; 2008: 11; pooled for analyses). Genomic DNA from each sample was extracted with the Invisorb Spin Swab Kit (Invitek, Berlin) according to the manufacturer's instructions. The DNA was resuspended in 100 µl Invitek Elution buffer and stored at -20°C. Eight species-specific microsatellite loci (WHA1-9, WHA1-60, WHA1-67, WHA1-104, WHA1-140, WHA1-20, WHA1-25, WHA1-103) previously isolated and characterised by Arens et al. (2000) were amplified, using procedures and protocols described in Krug & Proehl (2013). Genotyping was conducted using a capillary sequencer (MegaBace 1000, Amersham Bioscience) and resulting peaks were scored using Genetic Profiler v. 2.3. Allelic analyses were conducted using Genepop on the Web (Rousset 2008).

Three different statistical methods were applied to estimate the effective population size (N_e). The point estimator of Waples (1989), as incorporated in NeESTIMATOR (Peel et al. 2004), is based on variation in allele frequencies across generations and requires at least two samples. For *H. arborea*, the mean generation time was estimated at 2.7 years, based on an annual survival rate of 0.3, a mean number of reproductive years of 1.43, and the reaching of sexual maturity at the age of two years in both sexes (Tester 1990, Friedl & Klump 1997); thus the samples from 2005 and 2008 can be considered to stem from roughly two successive generations. Furthermore, two single-sample estimators were employed for both sampling years. LDNE (Waples & Do 2008), also incorporated in NeESTIMATOR, calculates N_e based on the gametic disequilibrium, reflecting non-random selection of parental gametes (Hill 1981, Waples 2006). A random mating system was chosen, and confidence intervals were generated by the jackknife method. P_{ecrit} was set to 0.05. All alleles with frequencies below the critical value were excluded from the analysis. The sibship assignment method (SA) also uses a single sample and was proposed by Wang (2009). It is based on the premise that N_e can be calculated by the number of half and full sibs found in a sample, and this can be estimated using the software COLONY (Jones & Wang 2010). The full likelihood model with medium precision was used without setting a sibship prior, allowing for polygamous mating in both sexes. Given the rather short generation time of *H. arborea*, the probability of false sibship assignments through parent-offspring relationships in the sample is low. The obtained N_e values also provided further information on the initial population size that was assumed for the PVAs by adopting known N_e/N values obtained from demographic data for *H. arborea* in a previous study (Broquet et al. 2009).

Population viability analyses

PVAs were performed with the software VORTEX 10 (Lacy & Pollak 2014), which allows the input of demographic as well as genetic data as described by Lacy (1993). A summary of input variables is shown in Table 1. For each scenario, a total of 1000 simulations were run and the time span was set to 50 years; extinction was defined as the absence of at least one sex. As an initial population size, we assumed 71 individuals based on two lines of evidence: field observations based on chorus counts (BUND/ABIA Hannover, unpubl. data), and a regressive calculation of population census size based on the obtained N_e estimates (see below) and previously published N_e/N values for *H. arbo-*

Table 1. Input parameters for the default scenario of the VORTEX simulation of a *Hyla arborea* population.

Variable	
No. of iterations	1000
No. of years	50
Duration of each year in days	365
Extinction definition	Only 1 sex remains
Lethal equivalents	6.29
% due to recessive lethals	50%
Reproductive system	Polygynous
Age of first offspring	2 years
Maximum breeding age	4 years
Sex ratio	1:1
% adult females breeding	80%
Mortality rate	75%
% males in breeding pool	100%
Density dependent reproduction	Not included
Specified age distribution	Yes
Initial population size	71
Carrying capacity	5000

rea (Broquet et al. 2009). However, as precise population size estimates as, for example, based on capture-recapture were unavailable, we also simulated a set of initial population sizes (27–92 individuals, based on results from the alternative N_e analyses). The sex ratio at birth was set to 1:1 (Pellet et al. 2006, Broquet et al. 2009). Conforming to Pellet et al. (2006), we assumed an age at maturity of two years for both sexes (although the value can differ across the species range, see Tester 1990, Giacoma et al. 1993, Friedl & Klump 1997). The maximum age of reproduction was set to four years (Stumpel & Hanekamp 1986, Giacoma et al. 1993). *Hyla arborea* has one reproductive season per year, during which females produce several consecutive clutches of eggs (Giacoma et al. 1993). Assuming a small proportion of reproductive failures, e.g., due to clutch desiccation, we set the mean number of yearly reproducing females at 80% (standard deviation of 20%). Due to a lack of data on egg and larval survival, we combined these two stages and expressed fecundity as first-year survivors (S_1) varying between 4.0 and 20.0 per female. We assumed an average clutch size of 400 eggs (Tester 1990, Baletto & Giacoma 1993, Grosse 1994, Broquet et al. 2009) and first-year survival rates between 0.01 and 0.05 (0.01 intervals). The breeding system was specified as polygynous, and the percentage of breeding males was set to 100% (males do not skip breeding seasons; Pellet et al. 2007). Annual adult mortality rates derived from recapture studies vary between 70 and 80% irrespective of age and sex (Tester 1990, Friedl & Klump 1997, Pellet et al. 2006), and we used the mean of 75%. Density-dependent reproduction was not included into the model, and the carrying capacity was set to a high value (5000), because it was assumed that the number of individuals able to occupy the pond is considerably higher than the observed numbers (pond sizes ca 900 and 1,500 m², respectively). A sensitivity analysis was performed by using alternative values of 1000, 2000, 3000 and 4000 individuals.

For incorporating genetic data into the PVA, we used starting allele frequencies derived from the empirical microsatellite data (see below). Small populations of *H. arborea* show evidence of inbreeding depression (reduced survival of early progeny, Edenhamn et al. 2000, Andersen et al. 2004). As recommended by Lacy & Pollak (2014), we therefore used a value of 6.29 lethal equivalents to simulate the effects of inbreeding on population persistence.

After calculating a baseline model, we also simulated the impact of a metapopulation structure on population survival rates, assuming two subpopulations characterized by the above-described population parameters. Dispersal in *H. arborea* is male- and juvenile-biased (Stumpel & Hanekamp 1986, Vos et al. 2000), and dispersal rates between adjacent ponds were reported as 6 and 9%, respectively (Stumpel & Hanekamp 1986). For the present simulation, we tested three scenarios: 10% dispersal for males, 5% dispersal for males, and 10% dispersal for male juveniles only. Dispersal mortality rates were assumed as 50% (default) and 30%. In addition to natural dispersal, we also modelled the effect of supplementation. Our goal was to identify the release stock number required to retain at least 90% of genetic diversity over 50 years (Frankham et al. 2010). A starting scenario was created where three males and females each are added to the population every three years, assuming allele frequencies from the putative propagule pool (data taken from Krug & Proehl 2013). Sensitivity tests were performed by varying the number of supplemented animals (2–8) as well as the time interval of supplementation (3–9 years).

Before the start of a simulation, VORTEX provides deterministic population growth rates (det-r) projected from life table calculations alone. This gives a first overview of whether the assumed rates of reproduction and survival allow for positive population growth in the absence of random fluctuations (Miller & Lacy 2005). Mean values of the following statistical parameters were documented at 5-year intervals: The mean stochastic growth rate (stoc-r), the mean population size (N_{all}) including both populations becoming extinct and those remaining extant; the probability of extinction (P_E) equal to the percentage of iterations that have become extinct, and mean observed and expected heterozygosities (H_o vs H_e).

Results

Basic genetic data and estimation of initial population size

The eight microsatellite loci employed proved moderately to highly polymorphic, and displayed between 4 and 7 alleles each (average 4.9, detailed data not shown). After applying Bonferroni correction, locus WHA1-60 displayed a significant excess of heterozygotes ($p < 0.01$), whereas all

Table 2. Genetic estimates of effective population size (N_e) for a *H. arborea* population; parentheses show 95% confidence limits. For the LD method, CIs were computed by jackknifing. Right column shows calculations of the initial population size (N_i) by using the effective breeding size (N_e) and a ratio provided by Broquet et al. (2009).

Method	Years covered	N_e	$N_i = N_e/0.48$
Point-estimator (Waples 1989)	2005–2008	33.9 (5.9–∞)	71
Linkage disequilibrium method (Waples & Do 2008)	2005	12.9 (4.3–77.9)	27
	2008	40.6 (8.8–∞)	85
Sibship assignment method (Wang 2009)	2005	23 (12–96)	48
	2008	44 (17–214743647)	92

other seven loci were in Hardy-Weinberg equilibriums (p ranging between 0.03 and 0.81).

Genetic estimates of N_e using three different methods are summarized in Table 2. The N_e value calculated by the temporal method lies within the single-sample calculations by the sibship assignment method. This was also the case for the LDNE method. Both single-sample estimators showed a higher N_e in 2008 in comparison to 2005. Based on the obtained values and previously published N_e/N values (Broquet et al. 2009), we assumed a most likely initial population size of 71 adults, which is in accordance with chorus counts (BUND/ABIA Hannover, unpubl. data).

Population viability analysis

The first series of simulations was run in order to estimate the effect of fecundity. Figure 1 presents the changes in mean population size (N_{all}) for different scenarios, with the mean final population sizes ranging between 1561 ($S_1 = 20$) and 0 ($S_1 = 4$). For all scenarios, the probability of extinction increased over the simulation period. An increase of first-year survival from 0.01 to 0.05 lowered the probability of extinction (P_E) by 46% after 50 years, with a sharp decrease for values above 0.02. Almost all simulated *H. arborea* populations became extinct at survival rates below 0.02 (Fig. 1). For further analyses, a first-year survival rate of 0.03 was used to provide a positive population growth based on life table calculations alone (det-r = 0.1, Appendix 1A). The mean stochastic growth rate for this scenario was slightly negative (stoc-r = -0.018), with high seasonal fluctuations (Appendix 1B). Sensitivity analysis showed that P_E varies between 0.82 and 0.88 depending on the method used to estimate N_e. The simulations were insensitive to the carrying capacity (Table 3).

VORTEX calculates the change in genetic variation as difference in expected heterozygosity (H_e) in the simulated time interval. The simulated populations lost on average 37% of the initial H_e after 50 years (see Appendix 2). Inbreeding depression through reduced survival increased the probability of population extinction by 16%, reducing the mean final population size N_{all} to 19.

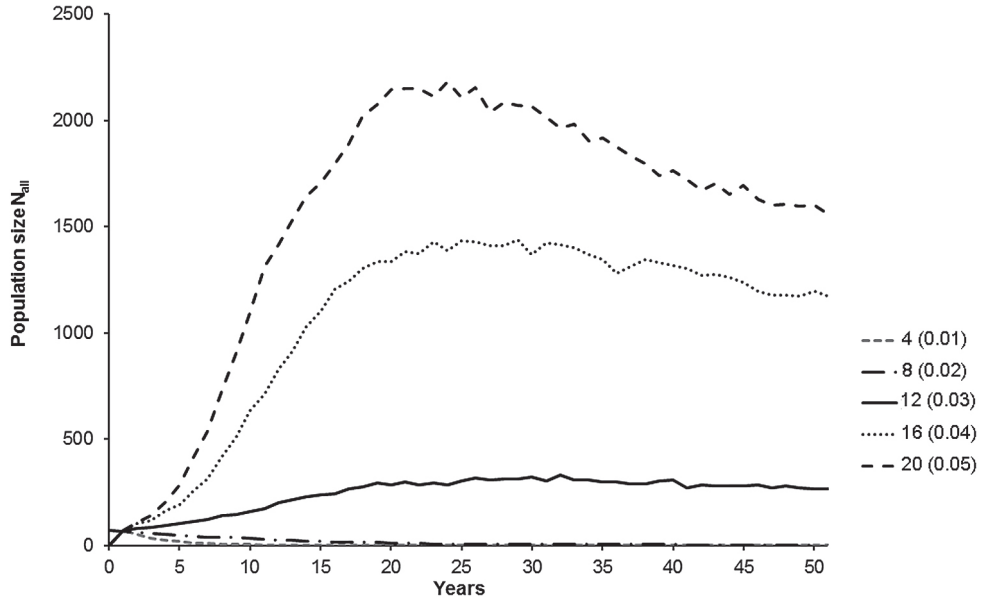

Figure 1. Relationship between time and mean population size (N_{all}) for varying numbers of offspring per female (S1) dependent on first-year survival rates (in brackets).

Table 3. Summary of output parameters. P_E = probability of extinction after 50 years, N_{all} = mean final population size, stoc-r = mean stochastic growth rate, S_1 = number of first-year survivors of offspring per female, N_i = initial population size, CC = carrying capacity, *default scenario.

Scenario	P_E	N_{all}	stoc-r
Default	0.83	265	-0.018
Sensitivity testing S_1			
4	1.0	0	-0.406
8	1.0	0	-0.174
12*	0.83	265	-0.018
16	0.60	1175	0.117
20	0.54	1561	0.202
Sensitivity testing N_i			
71*	0.83	265	-0.018
27	0.88	203	0.008
85	0.82	306	-0.016
48	0.87	199	-0.012
92	0.82	287	-0.017
Sensitivity testing CC			
1000	0.84	65	-0.014
2000	0.83	121	-0.02
3000	0.84	176	-0.019
4000	0.85	218	-0.018
5000*	0.83	275	-0.018
Inbreeding depression	0.98	19	-0.133
Metapopulation Model			
Metapop	0.65	646	-0.011
Subpop1	0.80	310	-0.010
Subpop2	0.80	337	-0.011

Figure 2A presents the probabilities of extinction for a modelled metapopulation with two demes. For both subpopulations, P_E was 80% after 50 years, and for the whole metapopulation, P_E was 65% with higher final population sizes compared to single-population simulations. A summary of results is given in Appendix 3. In general, values for all four models were similar, except that a migration rate set to 5% led to a higher probability of extinction, an effect which was less pronounced at reduced mortality rates and when dispersers were set to be exclusively juvenile males. When the population was supplemented with animals from the putative source population, P_E dropped to 44% (Fig. 3A) and the average population size approached 384 individuals. Mean genetic diversity (Fig. 3B) remained relatively stable, at increased H_o (0.56) and H_e (0.50), in comparison to the baseline model (see also Appendix 4). Sensitivity tests revealed that increasing the number of supplemental animals and reducing the time interval of supplementation markedly enhanced the population trajectory, without however any apparent threshold effect. The number of release stock necessary to retain at least 90% of genetic diversity was 8 individuals per generation (Table 4 and Appendix 4).

Table 4. Comparison of variations in output values when animals are supplemented. The default scenario assumes that six animals are supplemented (three males, three females) and that supplementation occurs every three years. Further models simulate changes by varying the number of supplemented animals (n) and time intervals of supplementation (Δa).

Scenario	P_E	N_{all}	stoc-r	Genetic diversity	No. of alleles
default: n=6, Δa=3	0.44	384	0.023	0.50	3.7
Sensitivity testing n					
2	0.75	301	0.02	0.44	3.2
4	0.59	326	0.027	0.48	3.44
8	0.30	546	0.024	0.53	3.93
Sensitivity testing Δa					
6	0.56	287	0.006	0.48	3.31
9	0.71	240	-0.009	0.42	2.97

Discussion

VORTEX is a computer simulation program that simulates the probability of extinction based on available biological parameters of the species modelled (Miller & Lacy 2005). In wildlife management, it serves primarily as a tool to identify the parameters with the highest influence on population trajectories to identify conservation strategies of high benefit. A major limitation of PVA is that parameters concerning the demography and life history of a species are sometimes hard to obtain, especially when the species is endangered (Boyce 1992). In this study, some demographic data were derived from the literature, including estimates based on populations from different parts of the species' range. Nevertheless, PVA is regarded as a powerful tool to manage threatened species even when concessions have to be made with respect to the demographic accuracy of input parameters (Brook et al. 2000, Olsen et al. 2014).

According to our knowledge, the present study is among the first to incorporate genetic data into a PVA conducted on anurans (for an example on urodeles, see Greenwald 2010). Our analyses showed that, in an isolated tree frog population, the future population size is highly sensitive to first-year survival rates, with low values leading to a high probability of population extinction over a 50-year period. This agrees with previous findings on other amphibians, which demonstrated that juvenile survival has a greater effect on population persistence than the survival rates of adults (e.g. *Pelobates fuscus*, Hels & Nachman 2002). The results of our PVA suggest that pond management measures to enhance early survival would markedly aid with population persistence, a finding that is in line with an empirical study on the effects of pond mitigation on population demography (see also van Buggenum & Vergoossen 2012).

Initial population sizes were chosen based on rough counts of calling males recorded from 1994–2014 (BUND/ABIA Hannover, unpubl. data), matching a value of 71 as derived from N_e values based on genetic data obtained

in 2005 and 2008 and previously published N_e/N values (Broquet et al. 2009). Sensitivity analysis revealed that there was no significant difference in the projected viability of the population based on the assumed range of initial population sizes (27–92). That all simulated populations will become extinct despite positive deterministic growth is likely due to high fluctuations in population sizes triggered by temporal variation in stochastic growth rates and high standard deviations in mean annual population sizes (a commonly observed phenomenon, see, e.g., Holsinger 2000). For *H. arborea*, stochastic interludes such as successive years with adverse conditions can indeed lead to rapid population extinction (Pellet et al. 2006). Small populations are more vulnerable to inbreeding and subsequent extinction (Boyce 1992, Frankham et al. 2010). Also, our outcome suggests that inbreeding depression can affect population survival in *H. arborea*. VORTEX allows modelling inbreeding depression as a reduction in first-

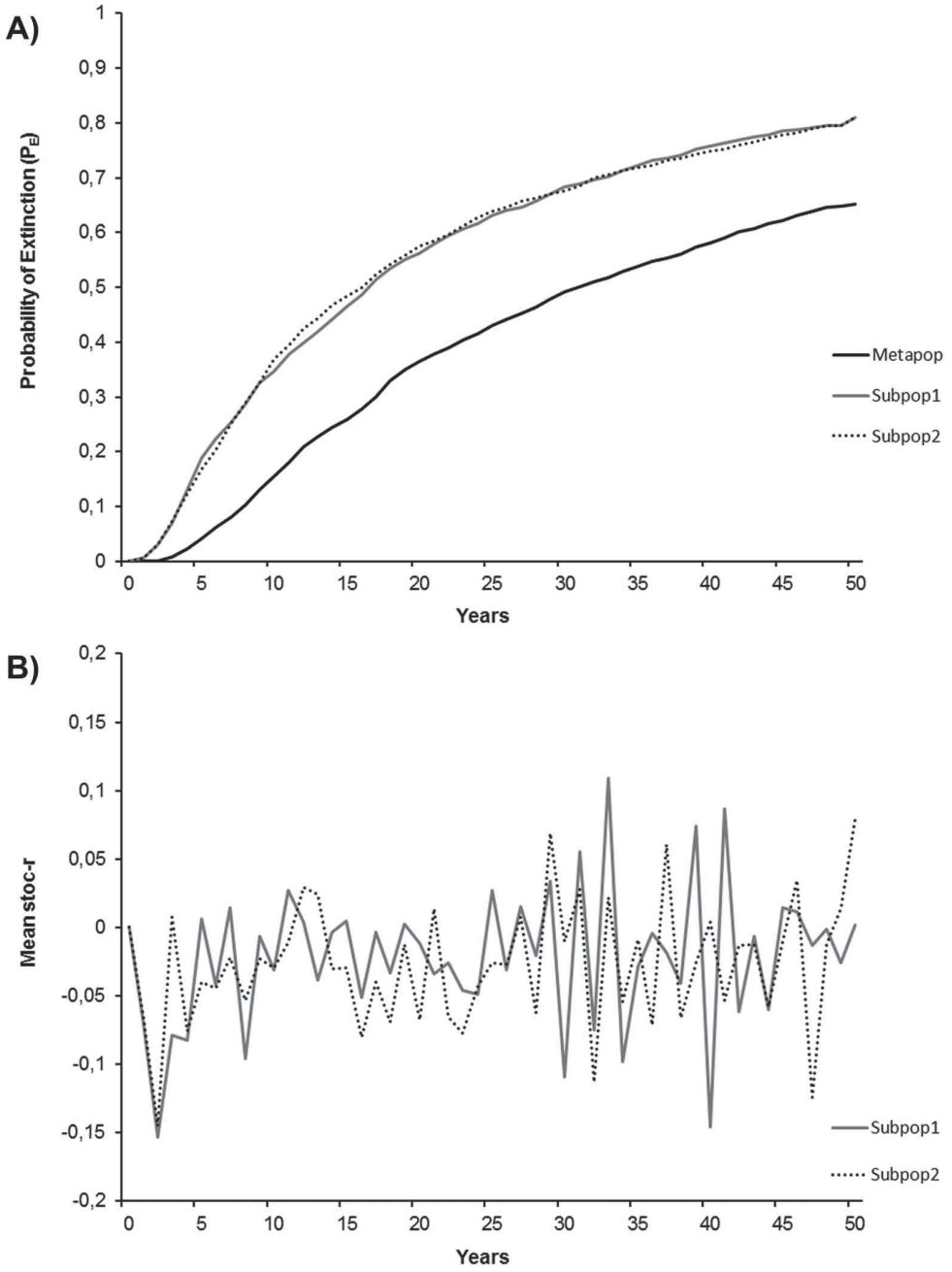

Figure 2. Metapopulation model assuming that all males are dispersing, a mortality rate of 50% for dispersers, and migration rates of 10% into both directions. (A) probability of extinction (P_E) over time, (B) annual fluctuation in mean stochastic growth rate (stoc-r) for both subpopulations.

year survival, but neglects other components of fitness possibly affected by inbreeding. However, our findings are in accordance with the negative relationship between inbreeding and tadpole survival found in wild *Rana sylvativa* populations (Halverson et al. 2006) and *H. arborea* under laboratory conditions (Luquet et al. 2011).

We assumed density-independence in survival and fecundity even though density-dependent responses of amphibian populations have previously been reported (Vonesh & De la Cruz 2002, Pellet et al. 2006). For example, Beebee et al. (1996) concluded for the natterjack toad, *Bufo calamita*, that adult population densities as well

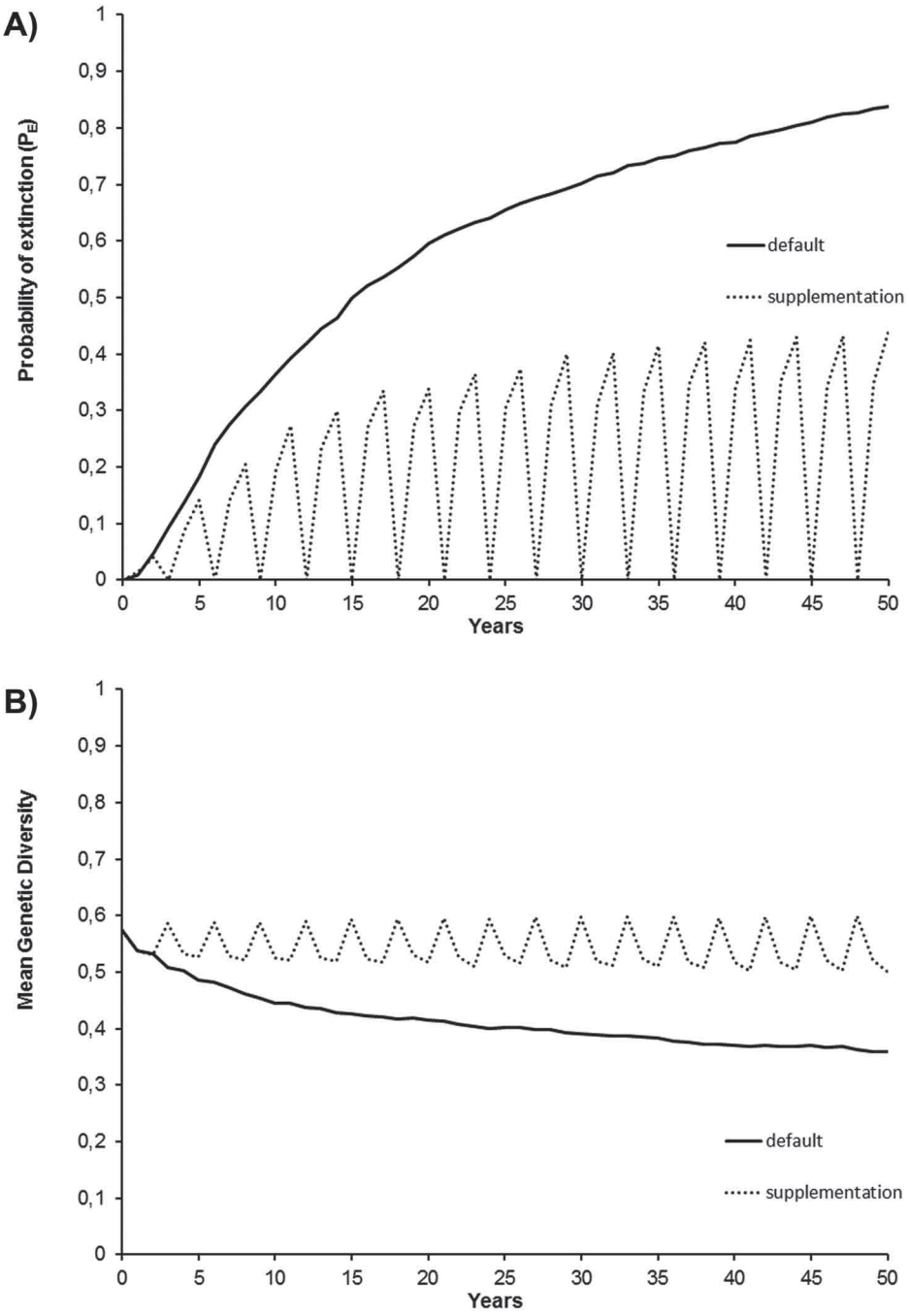

Figure 3. (A) Probability of extinction over time in the case of supplementation in comparison to the default scenario without supplementation. Here, six animals are supplemented every three years throughout the time frame; (B) shows mean genetic diversity over the years.

as regulation of toadlet production are related to stochastic rather than density-dependent processes, whereas population fluctuations in *H. arborea* were attributed to density-dependent and climatic factors (Pellet et al. 2006). However, caution has to be exercised when using a density-dependent model in a PVA (Boyce 1992, Brook et al. 1997), and we refrained from such an approach in the present study.

As *H. arborea* frequently occurs as metapopulations (Carlson & Edenhamn 2000, Pellet et al. 2006, Dubey et al. 2009, Krug & Proehl 2013), dynamic processes triggered by the exchange of individuals between populations (or the lack thereof) should be considered for conservation planning. When two connected demes were modelled, the survival time of the metapopulation exceeded those of the subpopulations, an effect that largely disappeared when dispersal rates were reduced to 5%. Hence, the rate and potential of migration should be considered as an important predictor for population persistence, and both are linked to *H. arborea* by its being subjected to high seasonal variation in reproductive success and availability of resources (e.g., Vos et al. 2000). A metapopulation is in danger of becoming extinct when all of its subpopulations are in decline, and that asynchrony in the demography of subpopulations is key to ensure persistence (e.g. this study, Hanski 1991) was confirmed with our simulations. *Hyla arborea* can exhibit source-sink processes (Carlson & Edenhamn 2000), and extinction events at single pond patches could be compensated by recolonisation.

The translocation of individuals to boost declining amphibian populations in a metapopulation context is highly controversial (see, e.g., Seigel & Dodd 2002, Marsh 2008, Germano & Bishop 2009). Positive aspects are the stabilization of population dynamics (Kinne 2005), but caution has to be taken especially regarding the transmission of parasites or diseases (Cunningham 1996). Since generally applicable protocols for translocations and supplementations are lacking (Germano et al. 2014, Sullivan et al. 2015), the PVA can be a helpful tool for facilitating decisions. Preserving 90% of an initial H_e is a suitable target for conservation strategies (Kaufman et al. 1993, Pertoldi et al. 2013). Our simulated supplementation of individuals from the putative source of the study population would facilitate that the latter's genetic diversity remained relatively stable over a period of 50 years, showing that the introduction of animals from a donor population could be an appropriate management tool.

Taken together, the persistence of the isolated *H. arborea* population appears to be largely governed by survival rates at premature stages as well as an influx from other demes (through metapopulation processes or by translocation and supplementation). Genetic erosion poses an additional risk, and could be compensated for through immigration even if the immigrant gene pool is similar to the focus population. Management strategies should entail ensuring a pond quality favourable to high survival rates of pre-metamorphic stages (such as the removal of introduced fish), and establishing new breeding ponds including the conservation of surrounding terrestrial habitat suitable for inter-pond migration. If the latter proves impossible due to constraints in available habitats, occasional supplementation will become desirable. We would anticipate that similar measures are also applicable to other *H. arborea* populations and other anuran species with similar life histories.

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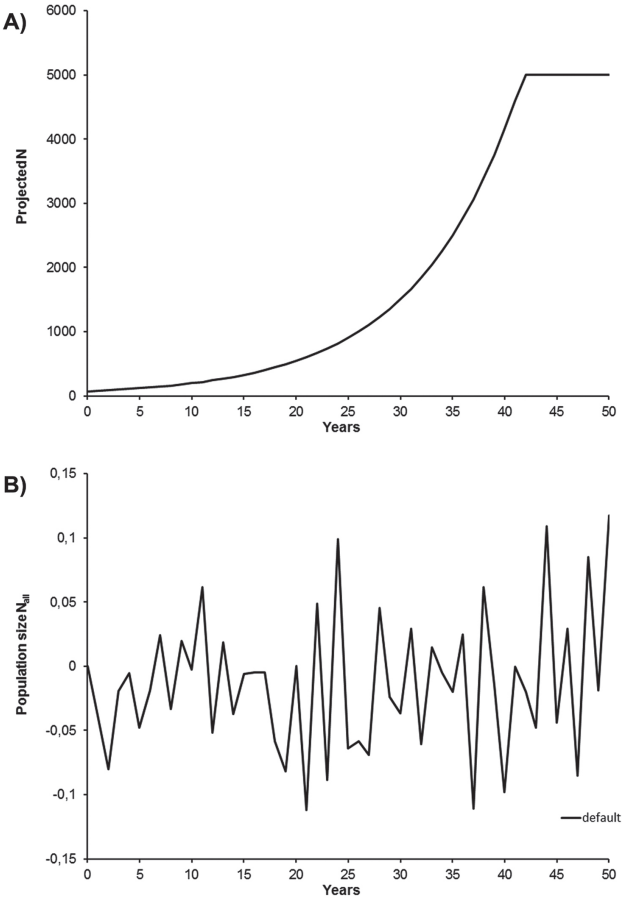
Appendix 1

(A) Deterministic projections of a baseline scenario, assuming a first-year survival rate of 0.03, no stochastic fluctuations, no inbreeding depression, and no limitation of mates (det-r = 0.1); (B) annual fluctuations in the mean stochastic growth rate (stoc-r) over time for the baseline scenario.



Appendix 2

Mean expected and observed heterozygosities (H_e/ H_o ± SD) for the basic scenario for each 5-year interval evaluated for simulations modelling allele frequencies.

Year	H_e (± SD)	H_o (± SD)
0	0.57 (± 0.01)	0.58 (± 0.02)
5	0.49 (± 0.08)	0.52 (± 0.08)
10	0.45 (± 0.10)	0.47 (± 0.10)
15	0.43 (± 0.11)	0.45 (± 0.11)
20	0.41 (± 0.12)	0.43 (± 0.12)
25	0.40 (± 0.12)	0.42 (± 0.12)
30	0.39 (± 0.12)	0.40 (± 0.12)
35	0.38 (± 0.12)	0.39 (± 0.12)
40	0.37 (± 0.13)	0.37 (± 0.13)
45	0.37 (± 0.13)	0.38 (± 0.12)
50	0.36 (± 0.13)	0.37 (± 0.12)

Appendix 3

Comparison of variations in the metapopulation model: (A) baseline model assuming that all males are dispersers, a migration rate of 10%, and a mortality rate of 50% amongst dispersers; further models simulate changes by varying (B) the pool of dispersers to exclusively juvenile males migrating; (C) the migration rate to 5%; and (D) the mortality rate amongst dispersers to 30%. N_{all}: mean final population size, P_E: mean probability of extinction, T_E: mean time to extinction.

Scenario	A			B			C			D		
	N_{all}	P_E	T_E	N_{all}	P_E	T_E	N_{all}	P_E	T_E	N_{all}	P_E	T_E
Metapop	646	0.65	22.0	581	0.68	23.0	463	0.70	22.5	597	0.68	21.9
Subpop 1	310	0.8	16.0	246	0.82	16.7	249	0.83	16.0	334	0.82	15.3
Subpop 2	337	0.8	15.8	335	0.81	15.2	215	0.83	15.5	262	0.81	15.9

Appendix 4

Mean expected and observed heterozygosities (H_e/ H_o ± SD) for each 5-year interval for a scenario where supplementation was included. The default scenario assumes that six animals (three males, three females) are supplemented and that supplementation occurs every three years. Further models simulate changes by varying the number of supplemented animals (n) and time interval of supplementation (Δa).

	Default scenario		Sensitivity testing n						Sensitivity testing Δa			
	n=6, Δa=3		2		4		8		6		9	
Year	H_e	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e	H_o
0	0.57 (± 0.01)	0.57 (± 0.02)	0.57 (± 0.01)	0.57 (± 0.02)	0.57 (± 0.01)	0.57 (± 0.02)	0.57 (± 0.01)	0.57 (± 0.02)	0.57 (± 0.01)	0.57 (± 0.01)	0.57 (± 0.01)	0.57 (± 0.02)
5	0.53 (± 0.08)	0.57 (± 0.10)	0.51 (± 0.08)	0.54 (± 0.09)	0.52 (± 0.07)	0.56 (± 0.09)	0.54 (± 0.08)	0.58 (± 0.10)	0.49 (± 0.08)	0.52 (± 0.08)	0.49 (± 0.07)	0.53 (± 0.08)
10	0.52 (± 0.08)	0.56 (± 0.11)	0.48 (± 0.09)	0.51 (± 0.11)	0.51 (± 0.08)	0.54 (± 0.11)	0.54 (± 0.08)	0.58 (± 0.11)	0.48 (± 0.09)	0.51 (± 0.10)	0.49 (± 0.10)	0.52 (± 0.13)
15	0.59 (± 0.08)	0.62 (± 0.12)	0.51 (± 0.08)	0.60 (± 0.15)	0.57 (± 0.08)	0.61 (± 0.13)	0.61 (± 0.08)	0.62 (± 0.11)	0.47 (± 0.10)	0.51 (± 0.13)	0.44 (± 0.11)	0.47 (± 0.11)
20	0.52 (± 0.09)	0.57 (± 0.12)	0.45 (± 0.10)	0.48 (± 0.12)	0.49 (± 0.09)	0.53 (± 0.12)	0.53 (± 0.09)	0.58 (± 0.12)	0.48 (± 0.10)	0.53 (± 0.14)	0.47 (± 0.11)	0.53 (± 0.16)
25	0.53 (± 0.09)	0.57 (± 0.13)	0.46 (± 0.11)	0.50 (± 0.15)	0.51 (± 0.10)	0.56 (± 0.14)	0.54 (± 0.09)	0.58 (± 0.12)	0.50 (± 0.11)	0.55 (± 0.15)	0.43 (± 0.12)	0.45 (± 0.12)
30	0.60 (± 0.09)	0.63 (± 0.12)	0.51 (± 0.08)	0.63 (± 0.16)	0.58 (± 0.09)	0.64 (± 0.14)	0.61 (± 0.09)	0.63 (± 0.11)	0.61 (± 0.09)	0.61 (± 0.09)	0.45 (± 0.11)	0.52 (± 0.17)
35	0.52 (± 0.09)	0.57 (± 0.13)	0.45 (± 0.11)	0.48 (± 0.13)	0.48 (± 0.10)	0.53 (± 0.14)	0.53 (± 0.09)	0.58 (± 0.11)	0.60 (± 0.10)	0.63 (± 0.14)	0.41 (± 0.12)	0.43 (± 0.13)
40	0.52 (± 0.10)	0.57 (± 0.13)	0.45 (± 0.10)	0.50 (± 0.15)	0.50 (± 0.10)	0.56 (± 0.15)	0.54 (± 0.09)	0.58 (± 0.12)	0.46 (± 0.10)	0.49 (± 0.12)	0.43 (± 0.11)	0.47 (± 0.14)
45	0.60 (± 0.09)	0.63 (± 0.13)	0.52 (± 0.08)	0.65 (± 0.16)	0.58 (± 0.09)	0.64 (± 0.14)	0.61 (± 0.09)	0.63 (± 0.11)	0.45 (± 0.10)	0.52 (± 0.15)	0.61 (± 0.11)	0.65 (± 0.14)
50	0.50 (± 0.10)	0.56 (± 0.13)	0.44 (± 0.11)	0.47 (± 0.14)	0.48 (± 0.10)	0.54 (± 0.15)	0.53 (± 0.09)	0.59 (± 0.13)	0.48 (± 0.11)	0.55 (± 0.16)	0.42 (± 0.11)	0.46 (± 0.14)