Scale-dependent effects of terrestrial habitat on genetic variation in the great crested newt (*Triturus cristatus*)

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Abstract

Context

Terrestrial landscapes surrounding aquatic habitat influence the persistence of amphibian spatially structured populations (SSPs) via their crucial role in providing estivation and overwintering sites, facilitating or hampering dispersal and colonisation, and consequently the maintenance or loss of genetic diversity.

Objectives

To highlight the landscape drivers of genetic variation, we investigated the relationship between the level of genetic variation measured within ponds of the great crested newt (*Triturus cristatus*), and the composition of the surrounding landscape at various spatial scales.

Methods

Based on the sampling of 40 ponds in thirteen SSPs, the influence of landscape features on several estimators of genetic variation was investigated via linear mixed models, with effects within and between SSPs incorporated.

Results

The best models depended on the spatial scale, with more significant associations within radii of 50 and 100 m of core ponds, particularly for allelic richness. Responses within and between SSPs were mostly similar. The availability of aquatic habitat in the landscape had a positive effect, while woodland, arable land and pasture had different effects depending on scale and response variable. Total length of roads within a 250 m radius influenced effective population size negatively.

Conclusions

Our results stress the need to investigate the influence of environmental predictors at multiple spatial scales for an adequate understanding of ongoing processes. Generally, the landscape affected genetic variation similarly within and between SSPs. This allowed us to provide general guidelines for the persistence of great crested newt populations, with an emphasis on the importance of the aquatic habitat.

Keywords

amphibians, genetic diversity, habitat fragmentation, spatial scales, *Triturus cristatus*

Introduction

Habitat loss and fragmentation due to human activities are the main causes of population declines and extinctions (Fischer 2000; Pimm and Raven 2000). They can lead to reduced gene flow among populations and decreasing population sizes with potentially negative consequences, such as an accelerated loss of genetic variation by genetic drift, inbreeding and an increasing risk of population extinction (Allendorf et al. 2013). As a population's adaptive and evolutionary potential is reflected in its level of genetic diversity (Frankham et al. 2017), it becomes important to learn how environmental factors might influence genetic variation.

Given their semi-aquatic life cycle, many amphibians occur in spatially structured populations. Spatially structured populations are typically described as a network of local populations that occupy discrete habitat patches connected by dispersing individuals (Thomas and Kunin 1999; Revilla and Wiegand 2008). The persistence of the spatially structured amphibian population does not only depend on the quality of the habitat sustaining the aquatic phase of the species, but also on the quality of the terrestrial habitat enabling estivating, overwintering, migration and dispersal. Terrestrial dispersal events include natal dispersal (from the natal site to the breeding site) and breeding dispersal (between breeding sites) (Semlitsch 2008). The configuration of the landscape matrix can therefore fundamentally affect connectivity of local populations (Van Buskirk 2005; Cayuela et al. 2020a). As the surrounding landscape plays an important role in dispersal, it is also involved in the maintenance or loss of genetic diversity (Hamer and McDonnell 2008). Gene flow (i.e. dispersal followed by reproduction, also termed effective dispersal) decreases genetic divergence among local populations and counters genetic drift (i.e. random loss due to a finite population size)

and inbreeding in local populations (Whitlock et al. 2000; Keller and Waller 2002). The loss of genetic diversity caused by genetic drift is inversely proportional to the effective population size (N_e). Consequently, already small populations will increasingly experience negative genetic effects under environmental pressures such as habitat destruction and pollution (Ralls et al. 2018).

So far, few studies directly investigated the relationship between habitat features and genetic variation within spatially structured amphibian populations (but see Curtis and Taylor 2004; Cosentino et al. 2012; Homola et al. 2019; Haugen et al. 2020). This should not be neglected as the habitat matrix might impose preconditions on genetic responses that are different from demographic observations. In addition, the spatial extent studied is also considered to play an important role (Cushman and McGarigal 2004; Galpern et al. 2012). Choosing a particular scale to identify landscape characteristics that influence populations can provide misleading predictions and leave landscape effects undetected (Jackson and Fahrig 2014). Although the number of studies using multiple geographical scales is steadily increasing, most of them have focussed on either abundance or presence/absence data (Gustafson et al. 2009; Hartel et al. 2010a; Denoël et al. 2013; Chambers et al. 2016; Macdonald et al. 2018; Boissinot et al. 2019), or proxies for gene flow such as genetic distances or data on migration (Johnson et al. 2002; Galpern et al. 2012; Coster et al. 2015; Cushman et al. 2016; Krishnamurthy et al. 2016; Zeller et al. 2017; Burgess and Garrick 2020; Winiarski et al. 2020).

Here, we studied thirteen spatially structured populations of the great (or northern) crested newt, *Triturus cristatus* (Laurenti, 1768). This amphibian species occurs throughout Europe (except southern Europe, where it is replaced by members of the same genus), and in parts of Western Siberia (Speybroeck et al. 2016). It receives EU-wide protection, being listed in Annexes II and IV of the European Habitats Directive (92/43/EEC 1992). As a result of significant population declines observed in monitoring programs and regional assessments, the species is red-listed in the majority

of countries and regions within its range (Dufresnes and Perrin 2015). Denoël (2012) found similar decline patterns in different countries and therefore suggested the species to be more susceptible than other newt species to environmental disturbance. Several studies exist on the effects of pond and terrestrial habitat characteristics on the abundance and occurrence of great crested newt, on its reproductive success, and on its dispersal behaviour (see e.g. Jehle and Arntzen 2000; Joly et al. 2001; Sztatecsny et al. 2004; Hartel et al. 2010b; Denoël et al. 2013; Vuorio et al. 2015; Miró et al. 2017; Denoël et al. 2018). In this study, we investigated the relationship between the level of genetic variation of great crested newts within breeding ponds, and the composition of the surrounding terrestrial landscape at various spatial scales using a hierarchical sampling design across multiple spatially structured populations. In addition to the multi-scale approach, several metrics estimating genetic variation, including effective population size, were used to provide overall and complementary information on the terrestrial landscape drivers of genetic variation. The specific aims were to investigate (1) which land cover types and features affect current genetic variation and effective population size of great crested newts at the pond-level, (2) if landscape components may have a different effect within and between spatially structured populations, (3) if scale affects these relationships and (4) at what scale genetic variation and effective population are most likely to be influenced by the terrestrial landscape.

Material and methods

Study sites, sampling and genotyping methods

The study area comprises thirteen spatially structured populations (SSP) of great crested newt located in different landscapes in Belgium. The majority of the sampled SSPs are situated in rural to peri-urban areas with agricultural fields, mainly consisting of pastures and arable land, and with urban elements surrounding the breeding ponds and SSPs. Human population density in Belgium is very high (377 people/km² in 2018; https://data.worldbank.org/indicator/EN.POP.DNST), especially in northern Belgium where the land surface is dominated by arable land and urban areas (Fig. 1, Fig.

S1). One to six ponds per SSP were sampled, delivering samples of 1179 adults, 58 metamorphosed juveniles and 299 larvae collected in 53 ponds in total (Fig. S1, Table S1).

DNA was extracted and genotyped at 31 microsatellite markers. Recaptured individuals were identified on the basis of the pattern on the newts' bellies and on their genotypes. Further potential bias in estimates of genetic variation can come from sampling close relatives due to the sampling design, such as sampling newt larvae in ponds (Goldberg and Waits 2010; O'Connell et al. 2019). Recaptures and all but one member per full-sib family were removed from the dataset for further analysis. We checked the remaining genotypes for the presence of null alleles, for possible deviations from Hardy-Weinberg equilibrium (HWE), and for linkage disequilibrium (LD) between pairs of loci. A more detailed description of the methodology can be found in Supplementary Material (Text S1, Tables S1 and S2).

After excluding low quality genotypes, recaptures and full sibs among larvae, genotypes of 1009 adults, 55 metamorphosed juveniles and 268 larvae remained, divided over 52 ponds in thirteen SSPs (Table S1). In the models to study the landscape effects on genetic variation we only included ponds with a minimum sample size of six individuals (see further) which reduces the number of studied ponds to 40 (Table S1 and S3).

Population genetics

In order to identify genetic clusters among SSPs and among breeding ponds within SSPs, we used the Bayesian program BAPS v. 6.0 (Corander et al. 2008). We performed 10 runs for each K = 1–40. An admixture analysis (Corander and Marttinen 2006) was performed using 100 iterations, a minimum of four individuals per population, 200 reference individuals for each population, and 20 iterations of reference individuals.

A second approach to assess population structure was used, Discriminant Analysis of Principal Components (DAPC) (Jombart et al. 2010) implemented in the R package adegenet 2.1.3 (Jombart 2008). Unlike BAPS, DAPC is independent from Hardy-Weinberg assumptions or linkage disequilibrium. We searched for clusters de novo by using the K-means procedure, with K = 1 – 60 with ten replicates per K. The best clustering model was selected based on the Bayesian Information Criterion (BIC). After an initial DAPC considering 150 principals components (PCs), the optimal number of PCs was chosen as the number PCs maximising the *a*-score. The *a*-score is the difference between the reassignment probability of the analysis and the reassignment probability obtained using random clusters.

Pairwise genetic differentiation F_{ST} (Weir and Cockerham 1984) among ponds, with a minimal sample size of six individuals, with corrected 95 % confidence intervals using 1000 bootstraps were calculated with the R package diveRsity 1.9.90 (Keenan et al. 2013). We further tested for differences in relatedness within and between ponds for each SSP with at least two sampled ponds with a minimum of six genotypes each using the DyadML coefficient (Milligan 2003) implemented in COANCESTRY 1.0.1.9 (Wang 2011). By randomly drawing dyads 1000 times while keeping the properties of the original groups, the average relatedness within ponds and difference in relatedness between ponds was then calculated each time, delivering a distribution with which the observed difference was compared. For each SSP and pond, observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness (A_R) and the inbreeding coefficient (F_{IS}) were estimated using the R package hierfstat 0.04-22 (Goudet 2005).

Estimates of effective population size (N_e) were calculated using the linkage disequilibrium method LDNe with a bias correction (Waples and Do 2008) implemented in NeEstimator 2.01 (Do et al. 2014) assuming random mating. The jackknife method was used to estimate 95 % confidence intervals across loci and a threshold for minimum allele frequency was set at 0.02 to further reduce bias

(Waples and Do 2010). Since the great crested newt is an iteroparous species and mostly mixed-age samples were collected, the number of effective breeders (N_b) refers to a mix of overlapping sets of parents in different years (Waples 2010). Waples et al. (2014) found that when a sample contains as many cohorts as there are in a generation, the estimated N_b approaches N_e. We also used a sibship assignment (SA) method implemented in the software COLONY 2.0.6.5 (Wang 2009; Jones and Wang 2010) to estimate N_e. Estimates from the software COLONY were based on the assumption of polygamous and random mating, and using the same settings as for the identification of full sibs among larvae were (Text S1). We did not include the estimates using only larval samples, which equal N_b for one reproductive cycle, because the majority of samples per pond contain mostly or exclusively samples of adults. To obtain values for N_e that are better comparable among ponds, we used only the genotypes of adults and metamorphosed juveniles for the calculations. For the SA method, we divided the samples into candidate mothers and fathers. Given the mixed ages of the adults, we included both adults and juveniles as potential offspring (Wang 2009; Wang and Santure 2009).

Landscape effects on genetic variation within and between SSPs

To assess the influence of the landscape surrounding the investigated ponds on the genetic variation, linear mixed models were built with estimates of A_R or F_{IS} as the response variable. H_E was highly correlated with A_R (Pearson's $r = 0.96$, $p < 0.0001$). We therefore chose not to use H_E as a response variable. We used estimates of N_e calculated with the LD- and SA-method as response variables as well. We used only estimates of N_e with finite 95% confidence limits as a response variable (log transformed).

Mixed effects models allowed us to analyse nested data (breeding ponds within SSPs). Based on the genetic structure results, no higher level of hierarchy had to be added to the models. Ponds with less than six samples were discarded.

As explanatory variables we derived land cover data from the map layer 'Bodembedekkingskaart' (BBK; Agentschap Informatie Vlaanderen (AIV)) created for Flanders with a resolution of 1 m. We used the editions for the situation in 2012 and 2015, as close to the two sampling periods as possible (2011-2012 and 2018, respectively). Additional landscape features were extracted from the map layer 'Grootschalig Referentie Bestand' (GRB) from the editions for 2014 and 2018 (AIV). Furthermore, a map layer with all known lentic features, 'Watervlakken version 1.0' (WV) (Packet et al. 2018), was used. Since these layers were developed for the region of Flanders, border locations such as De Panne (DP; close to the French border), Wervik (WR; close to the border with the Walloon region) and Marche-en-Famenne (MF; in the Walloon region) were not or not completely covered. We, therefore, used orthophotographs (summer images, 2012 edn., AIV) to complete the missing information for location WR, for which only a small percentage of the area was not covered by the Flemish maps, and for MF (2009-2010 edn., Service Public de Wallonie), while CES-OSO (Centre d'Expertise Scientifique sur l'occupation des sols) land cover map for France 2018 (Inglada et al. 2017) with a resolution of 10 m was used to derive land cover data for the missing part in location DP. To translate the land cover types in this map to those used in the layers BBK, GRB and WV, we assessed the overlapping parts together with the information obtained from orthophotos. Specifically for the location in question, on the North Sea Coast within the dunes, this resulted in transforming coniferous stands, according to CES-OSO, to grassland and shrubs, as defined by the BBK. Roads and railways in the coastal population were further identified on orthophotos and transformed to shapefiles in ArcGIS 10.4.1 (Esri 2016).

The effect of particular land cover types and features on genetic variation can be scale-dependent. For that reason, we derived landscape data within buffers of different sizes around each of the ponds, with a radius of 50, 100, 250 and 500 m. These distances were chosen according to the diversity of landscape components in the studied areas and to encompass distances commonly

crossed by great crested newts during migratory movements and dispersal to other breeding patches as well as during other terrestrial activities such as feeding, estivation, and overwintering (Miaud et al. 1993; Jehle 2000; Jehle and Arntzen 2000; Kupfer and Kneitz 2000). We did not include larger buffer sizes in the study due to the limited distances between the sampled ponds within the majority of the SSPs, ranging from 19 to 1702 m (mean = 327 m; Table S3). The following land cover classes were selected and calculated as the proportion of the investigated buffer area: natural grassland and shrubs, pastures, arable land, trees (small woodlands and forests) and surface water. Surface water included both lotic and lentic waters, since great crested newt was found in both ponds and some small canals, including the canal in ATG. However, this does not encompass larger lentic and lotic systems as none of these elements were present within the buffers used. The length of roads (all paved roads) was calculated as length (m) within a buffer. Distance to the nearest pond (m) was also included, which does not change with buffer size. The number of lentic systems within a buffer was also considered. The land cover types and landscape features are listed in Table S4. All operations to create buffers and to extract geospatial data from the maps within buffers were performed in the R environment (R Core Team 2019) using the packages raster 3.0-7 (Hijmans 2019), rgdal 1.4-8 (Bivand et al. 2019), rgeos 0.5-2 (Bivand and Rundel 2019), sp 1.3-1 (Pebesma and Bivand 2005; Bivand et al. 2013) and sf 0.8-0 (Pebesma 2018).

Adding a landscape component (which varies at the level of ponds) as explanatory variable may mask that the landscape component may have a different effect (in sign and/or strength) within versus between SSPs. Therefore, we used within-population centering to separate the within-SSP (W) effects from the between-SSP (B) effects (van de Pol and Wright 2009). The standard equation for a linear mixed model is given in Eq. 1, where x_{ij} is the pond-level covariate, u_{0j} the random intercept for SSP *j* and $\in_{ij}$ the pond-level residual.

$$y_{ij} = \beta_0 + \beta_1 x_{ij} + u_{0j} + \in_{ij} \qquad \text{(Eq. 1)}$$

$$\text{with } u_{0j} \sim N(0, \sigma^2_{u_{0j}}) \text{ and } \in_{ij} \sim N(0, \sigma^2_{\in_{ij}})$$

This can be changed to an equation with population-mean centered pond-level covariates, which expresses variation within SSP (β_W), and with population-mean ($\bar{x}_j$) added as a new covariate, which expresses the variation between SSPs (β_B) (Eq. 2).

$$y_{ij} = \beta_0 + \beta_W(x_{ij} - \bar{x}_j) + \beta_B(\bar{x}_j) + u_{0j} + \in_{ij} \quad \text{(Eq. 2)}$$

This adjustment to a standard mixed model allows us to test whether within-SSP and between-SSP effects are significantly different, by rearranging Eq. 2 (Eq. 3).

$$y_{ij} = \beta_0 + \beta_W x_{ij} + (\beta_B - \beta_W)\bar{x}_j + u_{0j} + \in_{ij} \quad \text{(Eq. 3)}$$

If the within-SSP and between-SSP effects for a certain variable are the same, then the population-mean is not included in the model (formulated according to Eq. 2).

We used the variance inflation factor (VIF) to evaluate the presence of multicollinearity, with a threshold of 3 to remove variables (Zuur and Ieno 2016). Since observations change with buffer size, the analysis was repeated for every buffer radius. Also, roads were only considered in the larger buffer sizes, starting at a radius of 250 m, as they were often absent in the smaller buffer sizes. The initial models did, therefore, not contain entirely the same set of land cover types as predictor variables. Still, we tried to create similar models with largely the same fixed predictor variables. Next, we used the *dredge* function of the R package MuMIn 1.43.17 (Bartón 2020) to find the best models with a ΔAICc of 2 or less than the top model. For this set of models, full model averaged coefficient estimates were calculated with their standard error, 95% confidence intervals, *Z*-values and *p*-values.

Results

Genetic diversity parameters for each microsatellite locus, averaged over ponds with at least six genotypes, are given in Table S5. Loci that were not in HWE after Bonferroni correction were locus TRCR427 in ponds 12s (BvA) and ZE6 (ZE), locus Tcri27 in pond PE (VK), loci Tcri29 and TRCR406 in pond P15 (ATG), and locus Tc50 in pond 12s (BvA). Proportions of null alleles were higher than 20%

for several loci mostly in one to three ponds within a single SSP (common results from GENEPOP and ML-NULLFREQ). Locus TRCR427 appeared to have null alleles in ZE and pond 12s (BvA).

Several pairs of loci appeared to be in LD (6.7 % of all pairs), of which seven pairs tested positive for LD at multiple ponds (between two and four ponds) with Bonferroni correction. After excluding larvae and reducing the number of adults to limit the chance of including families or multiple cohorts (Text S1), the remaining pairs of loci still exhibiting significant LD (3.2 % of all pairs) were limited to one pond.

Signals of Hardy-Weinberg disequilibria and null alleles seemed mostly SSP dependent. However, loci TRCR427 and TRCR406 came up multiple times in these tests, including those of LD detection. We therefore calculated F_{ST} values with and without these loci. As F_{ST} values hardly changed, we included both loci for further analysis.

Population genetics

The analysis using BAPS produced an optimal K of 15. This generally means that each SSP forms a separate cluster, but with a substructure in two SSPs (Fig. 2). Pond WR2 clustered separately from WR3 in SSP WR, and the majority of the samples in pond TE2 formed a separate cluster from the remaining samples of population TE. One individual of TE4 and one of TE5 were assigned to that cluster of TE2 and a few other samples showed some admixture between both genetic groups. The K-means results delivered the lowest mean BIC for K = 15 (mean BIC = 2862.8). However, similar BIC values were obtained for K between 12 and 17 groups (maximum mean BIC= 2865.2; Fig. S2). The DAPC results for K = 15 also clustered most SSPs separately, except for PE, with individuals assigned to the cluster containing TO and to the cluster containing DB (Fig. 2). Individuals of ponds TE2, WR2 and WR3 were not assigned to separate clusters, while SSPs WT and BvA were subdivided in two groups each, though not according to pond membership. Higher K values did also not always deliver

the same SSPs to be subdivided, while other SSPs were assigned to more than one cluster, again not according to their breeding patch (results not shown). The DAPC results for K =9 to 13 delivered no multiple clusters within populations, although for K = 14 the majority of the individuals of TE2 was assigned to a separate cluster, comparable to the BAPS results.

The SSPs were highly differentiated from each other (Fig. S3), even SSPs that are relatively near each other such as ZE and BvA (F_{ST} = 0.27, CI = 0.26 – 0.29). ATG is less genetically differentiated from VK than is expected based on the distance between them (F_{ST} = 0.07, CI = 0.06 – 0.08). Genetic differentiation estimates among ponds within SSPs were mostly small, though frequently significant (Fig. S3). In agreement with the clustering results, samples from pond WR2 were moderately differentiated from the samples from WR3 (F_{ST} = 0.09, CI = 0.07 – 0.12). Pond TE2 showed, however, a maximum among pond F_{ST} value of only 0.05 within TE.

Small but significant differences in relatedness within and among ponds were found in all of the SSPs tested, except in WT (Table S6). Relatedness within ponds was higher than among ponds, with differences ranging from 0.002 in WT to 0.05 in MH.

H_E values in ponds range from 0.43 to 0.71, A_R from 2.28 to 4.36 and F_{IS} from -0.15 to 0.11 (Table 1). Estimates of N_e using the LD-method and based on the genotypes of adult newts ranged from 6 to 502 (Table 1). This method often delivered confidence intervals with an infinite upper limit and in certain cases not even a finite estimate of N_e (in 47% of ponds with at least 6 adults). In contrast, 17% of the estimates of N_e calculated with the SA-method (ranging from 20 to 180) were not accompanied with finite confidence limits (Table 1). Both methods produced estimates (with finite confidence limits) that were comparable for those ponds exhibiting small N_e. Though, when LD-based N_e increased, SA-based estimates were often smaller.

Landscape effects on genetic variation within and between SSPs

The results of the top models (ΔAICc ≤ 2) for each response variable and each buffer size are presented in Table S7. The model-averaged parameters from these top models are shown in Table 2 and with more detailed information in Table S8. The predictors included in the models with significant parameter estimates after model averaging were mostly their within-SSP effects. This implies that the trends in the response variables explained by the landscape within SSPs did not differ from the trends among SSPs. Because we only included a random intercept, the slope of the relationship is the same in all SSPs, only the intercept differs. In general, the models using landscape variables within a radius of 50 and 100 m held the most significant covariates, although sometimes partly different ones depending on the buffer radius (Table 2 and S8).

The distance to the nearest pond showed a significant relationship with A_R on all scales, with a negative effect of increasing distances to the nearest pond (Fig. 3, Table 2). The estimate of the effect of this variable was similar for all buffer sizes. The proportion of surface water in a buffer showed a positive relationship with A_R, while an increase in the number of ponds and other lentic systems had a negative effect within SSPs on the same estimates within a radius of 50 or 100 m (Fig. 3a and b). As the number of lentic systems within these small buffer sizes was often limited to one to three ponds (Table S4), the number of populations showing a negative trend was small. Within a 100 m radius, the land covered by arable land had a positive within-SSP effect (Fig. 3b). The sign of this relationship changed when the buffer radius was 500 m (Fig. 3d). The influence of pastures on A_R was positive within a radius of 100 m (Fig. 3b), though only slightly significant (p = 0.03; Table 2 and S8).

With F_{IS} as response variable, the distance to the nearest pond was again included in the final model on all levels with negative estimates, although only significant on the smallest scale (p = 0.02; Table S8 and Fig. 4a). Within a radius of 50 m, the within-SSP effect of pastures was significantly negative

(Fig. 4a), as was the within-SSP effect of land covered by trees within radii of 100 and 250 m (Fig. 4b and c).

Sample size was reduced from 40 ponds in 13 SSPs to 30 ponds in 11 SSPs when N_e estimated with the SA-method was used as the response variable. On a scale of 100 m, the top models included a negative estimate for the within-SSP effect of the distance to the nearest pond and was significant after model averaging (p = 0.02; Table2 and S8, Fig. 5a). The length of roads within SSPs calculated within a radius of 250 m was a significant negative predictor (p = 0.02; Table 2 and S8, Fig. 5b). Average models obtained by using the LD-method N_e estimates as response variable showed no significant estimates, except for the proportion of land covered by trees within a buffer radius of 50 m (p = 0.01; Fig. 6, Table 2 and S8). Sample size was, however, reduced to 19 ponds within 11 populations.

Discussion

Using a multi-scale approach, we assessed the influence of the landscape surrounding breeding ponds on genetic diversity of the great crested newt. Our findings confirm the relevance and need to investigate the influence of environmental predictors on multiple spatial scales, as has been demonstrated in a growing number of ecological studies (e.g. Pellet et al. 2004a, 2004b; Hartel et al. 2010a; Angelone et al. 2011; Hamer and Parris 2011; Moraga et al. 2019). Such strategy is particularly meaningful, in terms of conservation, to highlight at which scale landscape features can affect patterns of distribution and genetic diversity. Moreover, the results offer new insights on how the terrestrial landscape may affect the genetic variation and effective population size. The land cover types studied here appeared to be mostly relevant on smaller spatial scales, within a radius of up to 100 m around breeding ponds. While the importance of the aquatic breeding habitat to sustain the populations' genetic variation and thus persistence was confirmed (Jehle et al. 2005; Karlsson et al. 2007; Rannap et al. 2009; Schön et al. 2011; Arntzen et al. 2017), the proportion of the buffer area

covered by trees, arable land, pasture and the presence of roads appeared to affect genetic measures variably, depending on scale and/or response variable.

Spatial scales and within- versus between-population effects

A population's history is known to significantly influence inferences on its current ecological processes (Anderson et al. 2010), especially past local extinction and (re)colonisation events (Wade and McCauley 1988). The landscape matrix of each population has its own history of change in habitat quality, amount and connectivity caused by environmental drivers such as land use change and invasive species (Wade and McCauley 1988; Kuussaari et al. 2009). Consequently, differences in landscape history and current composition as well as differences in past evolutionary processes within SSPs can lead to finding dissimilar relationships between genetic variation and current landscape features. Replicated empirical studies on the landscape scale are therefore warranted (Anderson et al. 2010). Instead of studying each population and landscape separately to obtain general conclusions (i.e. metareplication) (Johnson 2002), we addressed these issues by replacing the predictors by their within- and between-SSP effects. The difference in between- and within-SSP slope estimates was, however, in most models not significant, suggesting that the responses within and between SSPs were similar. Between-SSP effects still included in average models were often not significant, with the exception of wooded land cover within a radius of 50 m explaining the LD-based estimate of N_e. Here, the heterogeneous group of populations explained the between-SSP effect of the area covered by trees on the effective population size, which could not be clarified by the non-significant within-SSP predictor.

Complementarity of genetic responses

In general, the average models harbouring the most significant landscape variables were those for A_R. This response variable is considered to be particularly indicative of a population's resilience to change and its persistence (Caballero and García-Dorado 2013). It is therefore of high importance in

conservation and management practices. H_E and A_R respond differently to population changes. For instance, in case of a short genetic bottleneck, H_E may not change severely while the loss of alleles could be substantial (Allendorf 1986). Then, H_E favours common alleles while A_R takes rare alleles into account which tend to get lost more quickly. Particularly when considering heterozygosity measures, the current landscape might not entirely explain the observed levels of genetic response, due to genetic time lags (Epps and Keyghobadi 2015). Nevertheless, A_R and H_E, were highly correlated (r = 0.96) in our study. We therefore chose to use solely A_R as response variable to evaluate the effect of the current landscape on genetic variation.

As N_e is inversely proportional to the loss of genetic diversity caused by genetic drift, it can help predict or explain population genetic processes such as a reduction in genetic diversity, inbreeding and population differentiation (Weckworth et al. 2013). Although the LD approach has been proven to be robust (Gilbert and Whitlock 2015) and wide confidence intervals occur frequently for single-sample estimates (Luikart et al. 2010), many of our LD-based estimates of N_e were not accompanied with limited confidence intervals. Large confidence intervals are likely to occur when sample sizes are too small, especially when true N_e is large (Waples and Do 2010). The number of N_e estimates with finite confidence bounds were limited, reducing the sample size of the model considerably. Consequently, only one significant relationship between N_e and the between population effect of wooded land cover was found within a buffer radius of 50 m. This relationship was, however, not present in the models with SA-based N_e as the response variable. For both the LD- and SA-based estimates we used mixed-age samples. According to Waples et al. (2014), sampling as many cohorts as there are in a generation should deliver an LD-based estimate of N_e close to true N_e. Based on the data provided in recent studies performed by Cayuela et al. (2020b) and Orchard et al. (2019), mean generation length in the great crested newt ranged from 2.4 to 10 years and depended on both sex and population. Since the age of the sampled adults in our study is unknown, there is uncertainty if we achieved to sample the necessary number of different cohorts in every pond. Increased bias is

also expected when estimating N_e using a mixed-age sample with the SA-approach (Wang 2009; Wang and Santure 2009), although the number of markers in our study and their information content should be adequate to reduce bias (Wang 2009). Yet some bias is still expected due to low sample sizes in certain ponds. To obtain accurate SA-based N_e, sample sizes need to be near to or greater than true N_e (Ackerman et al. 2017). This condition was probably not met in those ponds with presumably larger true N_e.

F_{IS} is usually considered to express the level of inbreeding in a population. However, the values of F_{IS} were here mostly negative. This slight excess of heterozygotes could be caused by sampling an excessive proportion of relatives (Sánchez-Montes et al. 2017; Wang 2018). Although full sibs among larvae were excluded, close relatives among adults within a breeding pond could have been sampled, especially when the effective population size is small. Relatedness between individuals within ponds was significantly higher than among ponds within most of the studied SSPs, even in SSPs where ponds are very close to each other and F_{ST} values among ponds were low (e.g. TO). Because the majority of breeding patches are not in Hardy–Weinberg equilibrium, immigrants from other ponds will cause negative F_{IS} values to increase towards zero, rather than to a positive value. Instead of perceiving an increase in F_{IS} as an increase in inbreeding, we should therefore recognise it as an increase in immigration in our study. Still, not many significant explanatory variables were included in the models for F_{IS}, potentially because of its dependence on the composition of the samples making it difficult to standardise among ponds and SSPs.

Impact of the landscape

Across all buffer sizes, the distance to the nearest pond within-SSP was highly significant in the models explaining A_R. The negative relationship suggests that the closer the neighbouring pond the higher the chance of gene flow resulting in higher allelic diversity. In addition, a negative relationship with distance to the nearest pond appeared significant in the resulting model for F_{IS} within a radius of

50 m and for SA-based N_e within a radius of 100 m. Distance to the nearest pond appeared often to be an important connectivity metric to explain newt occurrence (Baker and Halliday 1999; Hartel et al. 2010b; Rannap et al. 2012; Miró et al. 2018). Denoël et al. (2018) further discovered a high rate of pond-infidelity among ponds within a few hundred meters in the great crested newt population of Marche-en-Famenne, also included in this study (MF). Consequently, a positive relationship between genetic diversity and the density of ponds was expected, since the number of ponds within 100 m also had a positive effect on great crested newt abundance (Denoël et al. 2013). However, the within-SSP effect of number of lentic systems was negative with A_R as response variable within buffer radii of 50 and 100 m. At these spatial scales, pond density was very low (one to three ponds) in many of the studied populations. Hence, this negative relationship appeared to be confined to a few populations and mainly steered by the situation in populations TO and MF where a variable number of ponds could be counted within such small buffer sizes. Moreover, the between-SSP effect of this landscape variable was not included in the initial models due to collinearity, which potentially could have delivered a different perspective. Moreover, pond characteristics and local hetero- and conspecific density were not taken into account, which are known to influence occupancy, breeding and immigration probability (Griffiths and Wijer 1994; Denoël and Ficetola 2008; Gustafson et al. 2009; Cayuela et al. 2018; Cayuela et al. 2019). Nevertheless, the proportion of area covered by surface water within populations affected A_R positively within the 50 m and 100 m radii. Surface water is in this study restricted to lentic and at some sites small lotic systems which include breeding sites of great crested newts. Lotic systems such as large rivers, which can act as barriers to gene flow (Maletzky et al. 2010), were not present within the buffers.

Great crested newts tend to migrate towards trees and forest edges (Jehle and Arntzen 2000; Malmgren 2002; Vuorio et al. 2015), where they may use forested areas as refuge and hibernation sites (Joly et al. 2001). However, we found a negative within-SSP effect of the proportion of the area covered by trees on F_{IS} within radii of 100 and 250 m. At close distances from ponds, an increasing

amount of wooded area could mean higher proportions of shoreline shading which is not preferred by great crested newts (Oldham et al. 2000; Maletzky et al. 2007). Shading can limit growth of macrophytes, which deliver many services to newts, and causes lower levels of dissolved oxygen due to reduced photosynthesis, and lower water temperature, which in turn slows down larval development. Sztatecsny et al. (2004) also found shading to be negative for larval abundance in great crested newts. Gustafson et al. (2011) used two scales in their study, a radius of 100 m and of 500 m, to examine the relationship of the terrestrial landscape and the presence of the species. Although they did not find a clear difference between scales in land use variables, they concluded that among other land uses, the abundance of old deciduous forest was important at the larger, landscape scale. This is supported by the positive effect across SSPs (i.e. between-SSP effect) of the proportion of land covered with trees on LD-based N_e, although within 50 m of the ponds.

Positive estimates for pasture area and arable land cover (within-SSP effects) were included in the top models explaining A_R at the 100 m scale. At this small scale, the benefits of an open landscape could outweigh potential negative effects. In addition, arable land is often scattered and infrequent within this buffer size. Hedgerows and ditches associated to pastures and arable fields can provide additional habitat and putative dispersal corridors. For instance, Jehle and Arntzen (2000) found post-breeding migration activity in newts associated with hedgerows whereas adjacent pastures were avoided. Within the 500 m radius, A_R appeared to be affected negatively by the within-SSP proportion of arable land. In addition, F_{IS} was negatively affected by the within-SSP pasture area within a 50 m radius. Arable land, pastures and rotation grasslands potentially deliver higher input of nutrients in nearby ponds. While adult great crested newts are able to thrive in aquatic habitats with a high nutrient content (Gustafson et al. 2009), eutrophication can speed up pond succession and potentially lead to toxicity (Egea-Serrano et al. 2011). Moreover, high levels of nutrients can negatively affect occurrence and reproduction of great crested newts (Gustafson et al. 2009; Denoël et al. 2013). Amphibians are also known to be sensitive to pesticides and fertilizer (Quaranta et al.

2009; Brühl et al. 2011), especially when exposed directly during migration (Berger et al. 2012; Lenhardt et al. 2015). Further research at the studied sites is, however, needed to ascertain the presence and importance of potential ecotoxicological risks related to arable land use.

In general, roads appear to influence amphibian populations negatively due to fragmentation or depletion effects (Fahrig et al. 1995; Gibbs and Shriver 2005; Jackson and Fahrig 2011). In our study, roads appeared with a significant and negative estimate in the 250 m radius model explaining SA-based N_e. According to Gustafson et al. (2011), great crested newts probably do not actively avoid roads, yet they are affected by road mortality (Matos et al. 2019). Some SSPs in our study are enclosed by roads, such as TO, which means that no roads need to be crossed to reach other breeding ponds within the SSP. However, some populations are intersected by minor roads, such as VK, TE and MF. Despite the low traffic densities on these roads, mortality risk during seasonal migration and dispersal increases with the number of roads within the habitat matrix (Matos et al. 2012; Matos et al. 2017).

Although a reasonable number of SSPs and ponds were sampled, the low number of ponds in several SSPs could be a potentially limiting factor to reliably identify landscape features that affect genetic variation. Particularly increasing the number of ponds within SSPs would produce a stronger within-SSP signal and improve statistical power to distinguish between potentially different effects within and between SSPs. Although more ponds were sampled, they could not be included in the models as a result of the low capture success or of low local abundance. Due to the elusive and rare character of the species in our study region, a larger number of ponds with a substantial number of individuals caught, could, however, only be achieved in a small number of SSPs. We would recommend extending the sampling coverage in the future by adding new data from other SSPs occurring in landscapes with similar components as included in this study. This would allow the sample size to increase without the loss of previously acquired information. In addition, future studies could benefit

from the use of a high number of genome-wide markers such as Single Nucleotide Polymorphisms (SNPs) to increase marker resolution and to investigate landscape influence on both neutral and adaptive variation (Balkenhol et al. 2019).

Conclusions

Although the scale of effect of the landscape can vary with the response variable (Moraga et al. 2019), the genetic response variables used here delivered models with significant predictors on mostly similar spatial scales. For great crested newts, genetic variation and effective population size as measured in individual ponds or demes, appear to be mainly influenced by the surrounding landscape on the smallest scales studied here (i.e. 50 – 100 m radius).

Since SSPs potentially went through different evolutionary processes and the surrounding terrestrial habitat is variable in quality and composition among SSPs, we expected different responses to the landscape within and among SSPs. Our approach eliminates the risk of mistakenly extrapolating from between-SSP effects to within-SSP effects or vice versa (van de Pol and Wright 2009). Results showed that, in general, the landscape variables studied here appeared to affect genetic variation within SSPs in a similar fashion as across SSPs. This allowed us to provide more general guidelines that would benefit the persistence of each great crested newt SSP.

Declarations

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Conflicts of interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

Consent to participate

Not applicable

Consent for publication

Not applicable

Data availability

Microsatellite genotypes were deposited in the Dryad repository under the accession doi.org/10.5061/dryad.573n5tb7v.

Code availability

Not applicable

Authors' contributions

KC, DA and GL conceived and designed the study. MD, DA, GL, SVdP and EP collected data and provided recourses. DH, LV and AVB performed the genetic analyses. KC performed the data analysis, with support from HVC, and wrote the first draft of the paper. All authors commented on previous versions of the manuscript, and read and approved the final manuscript.

References

92/43/EEC CD (1992) The conservation of natural habitats and of wild fauna and flora.

Ackerman MW, Hand BK, Waples RK, Luikart G, Waples RS, Steele CA, Garner BA, McCane J, Campbell MR (2017) Effective number of breeders from sibship reconstruction: empirical evaluations using hatchery steelhead. Evol Appl 10:146-160. https://doi.org/10.1111/eva.12433

Allendorf FW (1986) Genetic drift and the loss of alleles versus heterozygosity. Zoo Biol 5:181-190. https://doi.org/10.1002/zoo.1430050212

Allendorf FW, Luikart G, Aitken SN (2013) Genetics and the conservation of populations. Wiley-Blackwell Publishing, Oxford, UK

Anderson CD, Epperson BK, Fortin M-J, Holderegger R, James PMA, Rosenberg MS, Scribner KT, Spear S (2010) Considering spatial and temporal scale in landscape-genetic studies of gene flow. Mol Ecol 19:3565-3575. https://doi.org/10.1111/j.1365-294X.2010.04757.x

Angelone S, Kienast F, Holderegger R (2011) Where movement happens: scale-dependent landscape effects on genetic differentiation in the European tree frog. Ecography 34:714-722. https://doi.org/10.1111/j.1600-0587.2010.06494.x

Arntzen JW, Abrahams C, Meilink WRM, Iosif R, Zuiderwijk A (2017) Amphibian decline, pond loss and reduced population connectivity under agricultural intensification over a 38 year period. Biodivers Conserv 26:1411-1430. https://doi.org/10.1007/s10531-017-1307-y

Baker JMR, Halliday TR (1999) Amphibian colonization of new ponds in an agricultural landscape. Herpetol J 9:55-63.

Balkenhol N, Dudaniec RY, Krutovsky KV, Johnson JS, Cairns DM, Segelbacher G, Selkoe KA, von der Heyden S, Wang IJ, Selmoni O, Joost S (2019) Landscape genomics: Understanding relationships between environmental heterogeneity and genomic characteristics of populations. In: Rajora O. P. (ed), Population genomics: concepts, approaches and applications. Springer International Publishing, Cham, pp. 261-322

Bartón K (2020) MuMIn: multi-model inference. R package version 1.43.17.

Berger G, Graef F, Pfeffer H (2012) Temporal coincidence of migrating amphibians with mineral fertiliser applications on arable fields. Agric, Ecosyst Environ 155:62-69. https://doi.org/10.1016/j.agee.2012.04.006

Bivand R, Rundel C (2019) rgeos: interface to geometry engine - open source ('GEOS'). R package version 0.5-2.

Bivand R, Pebesma E, Gomez-Rubio V (2013) Applied spatial data analysis with R. Springer, NY

Bivand R, Keitt T, Rowlingson B (2019) rgdal: bindings for the 'geospatial' data abstraction library. R package version 1.4-8.

Boissinot A, Besnard A, Lourdais O (2019) Amphibian diversity in farmlands: Combined influences of breeding-site and landscape attributes in western France. Agric, Ecosyst Environ 269:51-61. https://doi.org/10.1016/j.agee.2018.09.016

Brühl CA, Pieper S, Weber B (2011) Amphibians at risk? Susceptibility of terrestrial amphibian life stages to pesticides. Environ Toxicol Chem 30:2465-72. https://doi.org/10.1002/etc.650

Burgess SM, Garrick RC (2020) Regional replication of landscape genetics analyses of the Mississippi slimy salamander, *Plethodon mississippi*. Landscape Ecol 35:337-351. https://doi.org/10.1007/s10980-019-00949-x

Caballero A, García-Dorado A (2013) Allelic diversity and its implications for the rate of adaptation. Genetics 195:1373-1384. https://doi.org/10.1534/genetics.113.158410

Cayuela H, Grolet O, Joly P (2018) Context-dependent dispersal, public information, and heterospecific attraction in newts. Oecologia 188:1069-1080. https://doi.org/10.1007/s00442-018-4267-3

Cayuela H, Schmidt BR, Weinbach A, Besnard A, Joly P (2019) Multiple density-dependent processes shape the dynamics of a spatially structured amphibian population. J Anim Ecol 88:164-177. https://doi.org/10.1111/1365-2656.12906

Cayuela H, Valenzuela-Sánchez A, Teulier L, Martínez-Solano Í, Léna J-P, Merilä J, Muths E, Shine R, Quay L, Denoël M, Clobert J, Schmidt BR (2020a) Determinants and consequences of dispersal in vertebrates with complex life cycles: A review of pond-breeding amphibians. Q Rev Biol 95:1-36. https://doi.org/10.1086/707862

Cayuela H, Griffiths RA, Zakaria N, Arntzen JW, Priol P, Léna J-P, Besnard A, Joly P (2020b) Drivers of amphibian population dynamics and asynchrony at local and regional scales. J Anim Ecol 89:1350-1364. https://doi.org/10.1111/1365-2656.13208

Chambers CL, Cushman SA, Medina-Fitoria A, Martínez-Fonseca J, Chávez-Velásquez M (2016) Influences of scale on bat habitat relationships in a forested landscape in Nicaragua. Landscape Ecol 31:1299-1318. https://doi.org/10.1007/s10980-016-0343-4

Corander J, Marttinen P (2006) Bayesian identification of admixture events using multilocus molecular markers. Mol Ecol 15:2833-2843. https://doi.org/10.1111/j.1365-294X.2006.02994.x

Corander J, Marttinen P, Siren J, Tang J (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinformatics 9:539. https://doi.org/10.1186/1471-2105-9-539

Cosentino BJ, Phillips CA, Schooley RL, Lowe WH, Douglas MR (2012) Linking extinction-colonization dynamics to genetic structure in a salamander metapopulation. P Roy Soc B-Biol Sci 279:1575-1582. https://doi.org/doi:10.1098/rspb.2011.1880

Coster SS, Babbitt KJ, Cooper A, Kovach AI (2015) Limited influence of local and landscape factors on finescale gene flow in two pond-breeding amphibians. Mol Ecol 24:742-758. https://doi.org/10.1111/mec.13062

Curtis JMR, Taylor EB (2004) The genetic structure of coastal giant salamanders (*Dicamptodon tenebrosus*) in a managed forest. Biol Conserv 115:45-54. https://doi.org/10.1016/S0006-3207(03)00092-2

Cushman SA, McGarigal K (2004) Patterns in the species–environment relationship depend on both scale and choice of response variables. Oikos 105:117-124. https://doi.org/10.1111/j.0030-1299.2004.12524.x

Cushman SA, Elliot NB, Macdonald DW, Loveridge AJ (2016) A multi-scale assessment of population connectivity in African lions (*Panthera leo*) in response to landscape change. Landscape Ecol 31:1337-1353. https://doi.org/10.1007/s10980-015-0292-3

Denoël M (2012) Newt decline in Western Europe: Highlights from relative distribution changes within guilds. Biodivers Conserv 21:2887-2898. https://doi.org/10.1007/s10531-012-0343-x

Denoël M, Ficetola GF (2008) Conservation of newt guilds in an agricultural landscape of Belgium: The importance of aquatic and terrestrial habitats. Aquat Conserv: Mar Freshwat Ecosyst 18:714-728. https://doi.org/10.1002/aqc.853

Denoël M, Perez A, Cornet Y, Ficetola GF (2013) Similar local and landscape processes affect both a common and a rare newt species. PloS One 8:e62727. https://doi.org/10.1371/journal.pone.0062727

Denoël M, Dalleur S, Langrand E, Besnard A, Cayuela H (2018) Dispersal and alternative breeding site fidelity strategies in an amphibian. Ecography 41:1543-1555. https://doi.org/10.1111/ecog.03296

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014) NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol Ecol Resour 14:209-214. https://doi.org/10.1111/1755-0998.12157

Dufresnes C, Perrin N (2015) Effect of biogeographic history on population vulnerability in European amphibians. Conserv Biol 29:1235-41. https://doi.org/10.1111/cobi.12490

Egea-Serrano A, Tejedo M, Torralva M (2011) Behavioral responses of the Iberian waterfrog, *Pelophylax perezi* (Seoane, 1885), to three nitrogenous compounds in laboratory conditions. Ecotoxicology 20:1246-1257. https://doi.org/10.1007/s10646-011-0673-6

Epps CW, Keyghobadi N (2015) Landscape genetics in a changing world: Disentangling historical and contemporary influences and inferring change. Mol Ecol 24:6021-6040. https://doi.org/10.1111/mec.13454

Esri (2016) ArcGIS Desktop. 10.4.1 edn. Environmental Systems Research Institute, Redlands, CA,

Fahrig L, Pedlar JH, Pope SE, Taylor PD, Wegner JF (1995) Effect of road traffic on amphibian density. Biol Conserv 73:177-182. https://doi.org/10.1016/0006-3207(94)00102-V

Fischer M (2000) Species loss after habitat fragmentation. Trends Ecol Evol 15:396. https://doi.org/10.1016/S0169-5347(00)01977-7

Frankham R, Ballou JD, Ralls K, Eldridge MDB, Dudash MR, Fenster CB, Lacy RC, Sunnucks P (2017) Genetic management of fragmented animal and plant populations. Oxford University Press, Oxford UK

Galpern P, Manseau M, Wilson P (2012) Grains of connectivity: Analysis at multiple spatial scales in landscape genetics. Mol Ecol 21:3996-4009. https://doi.org/10.1111/j.1365-294X.2012.05677.x

Gibbs JP, Shriver WG (2005) Can road mortality limit populations of pool-breeding amphibians? Wetlands Ecol Manage 13:281-289. https://doi.org/10.1007/s11273-004-7522-9

Gilbert KJ, Whitlock MC (2015) Evaluating methods for estimating local effective population size with and without migration. Evolution 69:2154-2166. https://doi.org/10.1111/evo.12713

Goldberg CS, Waits LP (2010) Quantification and reduction of bias from sampling larvae to infer population and landscape genetic structure. Mol Ecol Resour 10:304-313. https://doi.org/10.1111/j.1755-0998.2009.02755.x

Goudet J (2005) hierfstat, a package for r to compute and test hierarchical F-statistics. Mol Ecol Notes 5:184-186. https://doi.org/10.1111/j.1471-8286.2004.00828.x

Griffiths RA, Wijer Pd (1994) Differential effects of pH and temperature on embryonic development in the British newts (*Triturus*). J Zool 234:613-622. https://doi.org/10.1111/j.1469-7998.1994.tb04868.x

Gustafson DH, Andersen ASL, Mikusiński G, Malmgren JC (2009) Pond quality determinants of occurrence patterns of Great Crested Newts (*Triturus cristatus*). J Herpetol 43:300-310, 11.

Gustafson DH, Malmgren JC, Mikusiński G (2011) Terrestrial habitat predicts use of aquatic habitat for breeding purposes — a study on the Great Crested Newt (*Triturus cristatus*). Ann Zool Fenn 48:295-307. https://doi.org/10.5735/086.048.0504

Hamer AJ, McDonnell MJ (2008) Amphibian ecology and conservation in the urbanising world: A review. Biol Conserv 141:2432-2449. https://doi.org/10.1016/j.biocon.2008.07.020

Hamer AJ, Parris KM (2011) Local and landscape determinants of amphibian communities in urban ponds. Ecol Appl 21:378-390. https://doi.org/10.1890/10-0390.1

Hartel T, Schweiger O, Öllerer K, Cogălniceanu D, Arntzen JW (2010a) Amphibian distribution in a traditionally managed rural landscape of Eastern Europe: Probing the effect of landscape composition. Biol Conserv 143:1118-1124. https://doi.org/10.1016/j.biocon.2010.02.006

Hartel T, Nemes S, Öllerer K, CogăLniceanu DAN, Moga C, Arntzen JW (2010b) Using connectivity metrics and niche modelling to explore the occurrence of the northern crested newt *Triturus*

cristatus (Amphibia, Caudata) in a traditionally managed landscape. Environ Conserv 37:195-200. https://doi.org/10.1017/S037689291000055X

Haugen H, Linløkken A, Østbye K, Heggenes J (2020) Landscape genetics of northern crested newt *Triturus cristatus* populations in a contrasting natural and human-impacted boreal forest. Conserv Genet 21:515-530. https://doi.org/10.1007/s10592-020-01266-6

Hijmans RJ (2019) raster: geographic data analysis and modeling. R package version 3.0-7.

Homola JJ, Loftin CS, Kinnison MT (2019) Landscape genetics reveals unique and shared effects of urbanization for two sympatric pool-breeding amphibians. Ecol Evol 9:11799-11823. https://doi.org/10.1002/ece3.5685

Inglada J, Vincent A, Arias M, Tardy B, Morin D, Rodes I (2017) Operational high resolution land cover map production at the country scale using satellite image time series. Remote Sensing 9:95. https://doi.org/10.3390/rs9010095

Jackson ND, Fahrig L (2011) Relative effects of road mortality and decreased connectivity on population genetic diversity. Biol Conserv 144:3143-3148. https://doi.org/10.1016/j.biocon.2011.09.010

Jackson ND, Fahrig L (2014) Landscape context affects genetic diversity at a much larger spatial extent than population abundance. Ecology 95:871-881. https://doi.org/10.1890/13-0388.1

Jehle R (2000) The terrestrial summer habitat of radio-tracked great crested newts (*Triturus cristatus*) and marbled newts (*T. marmoratus*). Herpetol J 10:137-142.

Jehle R, Arntzen JW (2000) Post-breeding migrations of newts (*Triturus cristatus* and *T. marmoratus*) with contrasting ecological requirements. J Zool 251:297-306. https://doi.org/10.1111/j.1469-7998.2000.tb01080.x

Jehle R, Burke T, Arntzen JW (2005) Delineating fine-scale genetic units in amphibians: Probing the primacy of ponds. Conserv Genet 6:227-234. https://doi.org/10.1007/s10592-004-7832-8

Johnson CJ, Parker KL, Heard DC, Gillingham MP (2002) A multiscale behavioral approach to understanding the movements of woodland caribou. Ecol Appl 12:1840-1860. https://doi.org/10.1890/1051-0761(2002)012[1840:Ambatu]2.0.Co;2

Johnson DH (2002) The importance of replication in wildlife research. The Journal of Wildlife Management 66:919-932. https://doi.org/10.2307/3802926

Joly P, Miaud C, Lehmann A, Grolet O (2001) Habitat matrix effects on pond occupancy in newts. Conserv Biol 15:239-248. https://doi.org/10.1111/j.1523-1739.2001.99200.x

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551-555. https://doi.org/10.1111/j.1755-0998.2009.02787.x

Karlsson T, Betzholtz PE, Malmgren JC (2007) Estimating viability and sensitivity of the great crested newt *Triturus cristatus* at a regional scale. Web Ecol. 7:63-76. https://doi.org/10.5194/we-7-63-2007

Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA (2013) diveRsity: an R package for the estimation and exploration of population genetics parameters and their associated errors. Methods Ecol Evol 4:782-788. https://doi.org/10.1111/2041-210X.12067

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:230-241. https://doi.org/10.1016/S0169-5347(02)02489-8

Krishnamurthy R, Cushman SA, Sarkar MS, Malviya M, Naveen M, Johnson JA, Sen S (2016) Multi-scale prediction of landscape resistance for tiger dispersal in central India. Landscape Ecol 31:1355-1368. https://doi.org/10.1007/s10980-016-0363-0

Kupfer A, Kneitz S (2000) Population ecology of the great crested newt (*Triturus cristatus*) in an agricultural landscape: dynamics, pond fidelity and dispersal. Herpetol J 10:165-171.

Kuussaari M, Bommarco R, Heikkinen RK, Helm A, Krauss J, Lindborg R, Öckinger E, Pärtel M, Pino J, Rodà F, Stefanescu C, Teder T, Zobel M, Steffan-Dewenter I (2009) Extinction debt: A challenge for biodiversity conservation. Trends Ecol Evol 24:564-571. https://doi.org/10.1016/j.tree.2009.04.011

Lenhardt PP, Brühl CA, Berger G (2015) Temporal coincidence of amphibian migration and pesticide applications on arable fields in spring. Basic Appl Ecol 16:54-63. https://doi.org/10.1016/j.baae.2014.10.005

Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW (2010) Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. Conserv Genet 11:355-373. https://doi.org/10.1007/s10592-010-0050-7

Macdonald DW, Bothwell HM, Hearn AJ, Cheyne SM, Haidir I, Hunter LTB, Kaszta Ż, Linkie M, Macdonald EA, Ross J, Cushman SA (2018) Multi-scale habitat selection modeling identifies threats and conservation opportunities for the Sunda clouded leopard (*Neofelis diardi*). Biol Conserv 227:92-103. https://doi.org/10.1016/j.biocon.2018.08.027

Maletzky A, Kyek M, Goldschmid A (2007) Monitoring status, habitat features and amphibian species richness of Crested newt (*Triturus cristatus* superspecies) ponds at the edge of the species range (Salzburg, Austria). Ann. Limnol. - Int. J. Lim. 43:107-115. https://doi.org/10.1051/limn/2007017

Maletzky A, Kaiser R, Mikulíček P (2010) Conservation genetics of crested newt species *Triturus cristatus* and *T. carnifex* within a contact zone in Central Europe: impact of interspecific introgression and gene flow. Diversity 2:28-46. https://doi.org/10.3390/d2010028

Malmgren JC (2002) How does a newt find its way from a pond? Migration patterns after breeding and metamorphosis in great crested newts (*Triturus cristatus*) and smooth newts (*T. vulgaris*). Herpetol J 12:29-35.

Matos C, Sillero N, Argaña E (2012) Spatial analysis of amphibian road mortality levels in northern Portugal country roads. Amphibia-Reptilia 33:469. https://doi.org/10.1163/15685381-00002850

Matos C, Petrovan SO, Ward AI, Wheeler PM (2017) Facilitating permeability of landscapes impacted by roads for protected amphibians: patterns of movement for the great crested newt. PeerJ 5:e2922. https://doi.org/10.7717/peerj.2922

Matos C, Petrovan SO, Wheeler PM, Ward AI (2019) Landscape connectivity and spatial prioritization in an urbanising world: A network analysis approach for a threatened amphibian. Biol Conserv 237:238-247. https://doi.org/10.1016/j.biocon.2019.06.035

Miaud C, Joly P, Castanet J (1993) Variation in age structures in a subdivided population of *Triturus cristatus*. Canadian Journal of Zoology 71:1874-1879. https://doi.org/10.1139/z93-267

Milligan BG (2003) Maximum-Likelihood Estimation of Relatedness. Genetics 163:1153-1167.

Miró A, O'Brien D, Hall J, Jehle R (2017) Habitat requirements and conservation needs of peripheral populations: the case of the great crested newt (*Triturus cristatus*) in the Scottish Highlands. Hydrobiologia 792:169-181. https://doi.org/10.1007/s10750-016-3053-7

Miró A, Sabás I, Ventura M (2018) Large negative effect of non-native trout and minnows on Pyrenean lake amphibians. Biol Conserv 218:144-153. https://doi.org/10.1016/j.biocon.2017.12.030

Moraga AD, Martin AE, Fahrig L (2019) The scale of effect of landscape context varies with the species' response variable measured. Landscape Ecol 34:703-715. https://doi.org/10.1007/s10980-019-00808-9

O'Connell KA, Mulder KP, Maldonado J, Currie KL, Ferraro DM (2019) Sampling related individuals within ponds biases estimates of population structure in a pond-breeding amphibian. Ecol Evol 9:3620-3636. https://doi.org/10.1002/ece3.4994

Oldham RS, Keeble J, Swan MJS, Jeffcote M (2000) Evaluating the suitability of habitat for the great crested newt (*Triturus cristatus*). Herpetol J 10:143-155.

Orchard D, Tessa G, Jehle R (2019) Age and growth in a European flagship amphibian: equal performance at agricultural ponds and favourably managed aquatic sites. Aquat Ecol 53:37-48. https://doi.org/10.1007/s10452-018-09671-3

Packet J, Scheers K, Smeekens V, Leyssen A, Wils C, Denys L (2018) Watervlakken versie 1.0: polygonenkaart van stilstaand water in Vlaanderen. Een nieuw instrument voor onderzoek, water-, milieu- en natuurbeleid. Rapporten van het Instituut voor Natuur- en Bosonderzoek 2018. Instituut voor Natuur- en Bosonderzoek, Brussel,

Pebesma E (2018) Simple features for R: standardized support for spatial vector data. The R Journal 10:439-446. https://doi.org/10.32614/RJ-2018-009

Pebesma E, Bivand R (2005) Classes and methods for spatial data in R. R News 5

Pellet J, Guisan A, Perrin N (2004a) A concentric analysis of the impact of urbanization on the threatened European tree frog in an agricultural landscape. Conserv Biol 18:1599-1606. https://doi.org/10.1111/j.1523-1739.2004.0421a.x

Pellet J, Hoehn S, Perrin N (2004b) Multiscale determinants of tree frog (*Hyla arborea* L.) calling ponds in western Switzerland. Biodiversity & Conservation 13:2227-2235. https://doi.org/10.1023/B:BIOC.0000047904.75245.1f

Pimm SL, Raven P (2000) Extinction by numbers. Nature 403:843-845. https://doi.org/10.1038/35002708

Quaranta A, Bellantuono V, Cassano G, Lippe C (2009) Why amphibians are more sensitive than mammals to xenobiotics. PLoS One 4:e7699. https://doi.org/10.1371/journal.pone.0007699

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria,

Ralls K, Ballou JD, Dudash MR, Eldridge MDB, Fenster CB, Lacy RC, Sunnucks P, Frankham R (2018) Call for a paradigm shift in the genetic management of fragmented populations. Conserv Lett 11:e12412. https://doi.org/https://doi.org/10.1111/conl.12412

Rannap R, Lõhmus A, Briggs L (2009) Restoring ponds for amphibians: a success story. Hydrobiologia 634:87-95. https://doi.org/10.1007/s10750-009-9884-8

Rannap R, Lõhmus A, Linnamägi M (2012) Geographic variation in habitat requirements of two coexisting newt species in Europe. Acta Zool Acad Sci Hung 58:69-86.

Revilla E, Wiegand T (2008) Individual movement behavior, matrix heterogeneity, and the dynamics of spatially structured populations. P Natl Acad Sci USA 105:19120-19125. https://doi.org/10.1073/pnas.0801725105

Sánchez-Montes G, Ariño AH, Vizmanos JL, Wang J, Martínez-Solano Í (2017) Effects of sample size and full sibs on genetic diversity characterization: A case study of three syntopic Iberian pond-breeding amphibians. J Hered 108:535-543. https://doi.org/10.1093/jhered/esx038

Schön I, Raepsaet A, Goddeeris B, Bauwens D, Mergeay J, Vanoverbeke J, Martens K (2011) High genetic diversity but limited gene flow in Flemish populations of the crested newt, *Triturus cristatus*. Belg J Zool 141:3-13.

Semlitsch RD (2008) Differentiating migration and dispersal processes for pond-breeding amphibians. The Journal of Wildlife Management 72:260-267. https://doi.org/10.2193/2007-082

Speybroeck J, Beukema W, Bok B, Van Der Voort J, Velikov I (2016) Field guide to the amphibians and reptiles of Britain and Europe. Boomsbury Publishing, London

Sztatecsny M, Jehle R, Schmidt BR, Arntzen JW (2004) The abundance of premetamorphic newts (*Triturus cristatus*, *T. marmoratus*) as a function of habitat determinants : an *a priori* model selection approach. Herpetol J 14:89-97. https://doi.org/10.5167/uzh-336

Thomas CD, Kunin WE (1999) The spatial structure of populations. J Anim Ecol 68:647-657. https://doi.org/10.1046/j.1365-2656.1999.00330.x

Van Buskirk J (2005) Local and landscape influence on amphibian occurrence and abundance. Ecology 86:1936-1947. https://doi.org/10.1890/04-1237

van de Pol M, Wright J (2009) A simple method for distinguishing within- versus between-subject effects using mixed models. Anim Behav 77:753-758. https://doi.org/10.1016/j.anbehav.2008.11.006

Vuorio V, Tikkanen O-P, Mehtätalo L, Kouki J (2015) The effects of forest management on terrestrial habitats of a rare and a common newt species. Eur J Forest Res 134:377-388. https://doi.org/10.1007/s10342-014-0858-7

Wade MJ, McCauley DE (1988) Extinction and recolonization: their effects on the genetic differentiation of local populations. Evolution 42:995-1005. https://doi.org/10.1111/j.1558-5646.1988.tb02518.x

Wang J (2009) A new method for estimating effective population sizes from a single sample of multilocus genotypes. Mol Ecol 18:2148-2164. https://doi.org/10.1111/j.1365-294X.2009.04175.x

Wang J (2011) COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol Ecol Resour 11:141-5. https://doi.org/10.1111/j.1755-0998.2010.02885.x

Wang J (2018) Effects of sampling close relatives on some elementary population genetics analyses. Mol Ecol Resour 18:41-54. https://doi.org/10.1111/1755-0998.12708

Wang J, Santure AW (2009) Parentage and sibship inference from multilocus genotype data under polygamy. Genetics 181:1579-1594. https://doi.org/10.1534/genetics.108.100214

Waples RS (2010) Spatial-temporal stratifications in natural populations and how they affect understanding and estimation of effective population size. Mol Ecol Resour 10:785-796. https://doi.org/10.1111/j.1755-0998.2010.02876.x

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Resour 8:753-756. https://doi.org/10.1111/j.1755-0998.2007.02061.x

Waples RS, Do C (2010) Linkage disequilibrium estimates of contemporary N e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evol Appl 3:244-62. https://doi.org/10.1111/j.1752-4571.2009.00104.x

Waples RS, Antao T, Luikart G (2014) Effects of overlapping generations on linkage disequilibrium estimates of effective population size. Genetics 197:769-780. https://doi.org/10.1534/genetics.114.164822

Weckworth BV, Musiani M, DeCesare NJ, McDevitt AD, Hebblewhite M, Mariani S (2013) Preferred habitat and effective population size drive landscape genetic patterns in an endangered species. Proc R Soc Lond, Ser B: Biol Sci 280https://doi.org/10.1098/rspb.2013.1756

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370. https://doi.org/10.2307/2408641

Whitlock MC, Ingvarsson PK, Hatfield T (2000) Local drift load and the heterosis of interconnected populations. Heredity 84:452-457. https://doi.org/10.1046/j.1365-2540.2000.00693.x

Winiarski KJ, Peterman WE, Whiteley AR, McGarigal K (2020) Multiscale resistant kernel surfaces derived from inferred gene flow: An application with vernal pool breeding salamanders. Mol Ecol Resour 20:97-113. https://doi.org/10.1111/1755-0998.13089

Zeller KA, Vickers TW, Ernest HB, Boyce WM (2017) Multi-level, multi-scale resource selection functions and resistance surfaces for conservation planning: Pumas as a case study. PLoS One 12:e0179570. https://doi.org/10.1371/journal.pone.0179570

Zuur AF, Ieno EN (2016) A protocol for conducting and presenting results of regression-type analyses. Methods Ecol Evol 7:636-645. https://doi.org/10.1111/2041-210x.12577

Tables

Table 1 Genetic diversity and N_e estimates of the great crested newt spatially structured populations and ponds when the sample size exceeds five. The standard error for A_R, H_E, H_O and F_{IS}, and 95% confidence intervals for N_e esitmates are given in parentheses

SPP-pond	N	Na	H_O	H_E	F_{IS}	A_R	LD-N_e	SA-N_e
ATG	52	52	0.56 (0.04)	0.62 (0.04)	0.09 (0.05)	4.52 (0.38)		
ATG-P15	32	32	0.54 (0.05)	0.61 (0.04)	0.12 (0.05)	3.59 (0.25)	30 (25—38)	34 (22—59)
BvA	190	190	0.47 (0.05)	0.45 (0.04)	-0.04 (0.06)	2.61 (0.38)		
BvA-12l	37	37	0.49 (0.06)	0.43 (0.04)	-0.13 (0.06)	2.28 (0.15)	168 (49—∞)	30 (17—52)
BvA-12s	84	84	0.46 (0.05)	0.45 (0.04)	-0.02 (0.07)	2.37 (0.15)	69 (37—181)	40 (26—63)
BvA-4A	66	66	0.48 (0.05)	0.46 (0.04)	-0.03 (0.05)	2.46 (0.16)	74 (43—184)	31 (19—53)
DB	101	96	0.71 (0.04)	0.69 (0.03)	-0.02 (0.04)	5.52 (0.39)		
DB-DB15	10	10	0.72 (0.04)	0.71 (0.03)	-0.01 (0.04)	4.36 (0.27)	∞ (273—∞)	180 (50—∞)
DB-DB2	14	13	0.71 (0.04)	0.68 (0.04)	-0.06 (0.05)	4.18 (0.29)	439 (72—∞)	62 (28—∞)
DB-DB3	54	52	0.71 (0.04)	0.69 (0.03)	-0.03 (0.03)	4.15 (0.23)	81 (68—98)	71 (50—108)
DB-DB5	23	21	0.69 (0.05)	0.64 (0.04)	-0.07 (0.04)	3.89 (0.24)	19 (16—22)	32 (18—62)
DP	55	31	0.57 (0.05)	0.55 (0.04)	-0.06 (0.05)	3.75 (0.33)		
DP-DP1	6	6	0.49 (0.06)	0.52 (0.05)	0.06 (0.08)	3.68 (0.54)	6 (3—16)	30 (10—∞)
DP-DP3	36	12	0.58 (0.05)	0.53 (0.04)	-0.11 (0.05)	3.00 (0.22)	∞ (57—∞)	66 (25—∞)
MF	168	168	0.63 (0.04)	0.60 (0.04)	-0.06 (0.04)	4.23 (0.34)		
MF-MFH4	29	29	0.62 (0.05)	0.59 (0.04)	-0.04 (0.06)	3.37 (0.23)	364 (119—∞)	62 (36—111)
MF-MFH7	30	30	0.65 (0.04)	0.60 (0.04)	-0.10 (0.05)	3.42 (0.23)	148 (85—467)	64 (40—114)
MF-MFH9	20	20	0.62 (0.05)	0.59 (0.04)	-0.03 (0.06)	3.49 (0.25)	∞ (299—∞)	63 (35—157)
MF-MFJ22	29	29	0.65 (0.04)	0.61 (0.04)	-0.07 (0.05)	3.48 (0.23)	481 (143—∞)	71 (43—133)
MF-MFJ33	30	30	0.62 (0.04)	0.59 (0.04)	-0.07 (0.05)	3.42 (0.24)	454 (144—∞)	60 (38—102)
MF-MFK2	30	30	0.61 (0.05)	0.60 (0.04)	-0.01 (0.06)	3.50 (0.26)	502 (94—∞)	54 (34—94)
MH	56	56	0.51 (0.05)	0.48 (0.04)	-0.06 (0.05)	3.04 (0.23)		
MH-Stad1	38	38	0.51 (0.05)	0.46 (0.04)	-0.10 (0.06)	2.55 (0.18)	26 (20—35)	24 (14—45)
MH-Stad2	11	11	0.49 (0.05)	0.46 (0.04)	-0.07 (0.06)	2.59 (0.18)	58 (20—∞)	20 (9—59)
PE-PE0	6	0	0.65 (0.05)	0.58 (0.04)	-0.15 (0.06)	3.06 (0.22)		
TE	210	113	0.58 (0.05)	0.56 (0.05)	-0.03 (0.05)	4.38 (0.45)		
TE-TE1	29	12	0.56 (0.05)	0.56 (0.05)	-0.01 (0.05)	3.43 (0.29)	∞ (∞—∞)	33 (16—141)
TE-TE2	61	40	0.54 (0.06)	0.52 (0.05)	-0.05 (0.05)	3.11 (0.28)	26 (22—33)	47 (30—76)
TE-TE4	106	59	0.60 (0.05)	0.56 (0.05)	-0.07 (0.05)	3.43 (0.29)	188 (132—317)	83 (59—117)
TE-TE5	14	2	0.61 (0.06)	0.56 (0.05)	-0.09 (0.06)	3.43 (0.32)		
TO	123	40	0.68 (0.04)	0.65 (0.03)	-0.04 (0.04)	5.12 (0.44)		
TO-TO0	9	9	0.71 (0.04)	0.65 (0.03)	-0.11 (0.05)	3.96 (0.33)	∞ (246—∞)	∞ (1—∞)
TO-TO1	14	5	0.65 (0.05)	0.64 (0.04)	-0.01 (0.06)	3.84 (0.28)		
TO-TO2	11	6	0.69 (0.04)	0.64 (0.03)	-0.09 (0.05)	3.84 (0.27)	∞ (∞—∞)	∞ (1—∞)
TO-TO3	17	8	0.71 (0.05)	0.63 (0.04)	-0.10 (0.04)	3.92 (0.29)	∞ (∞—∞)	31 (14—158)
TO-TO4	34	11	0.69 (0.04)	0.66 (0.03)	-0.04 (0.04)	3.94 (0.26)	∞ (107—∞)	55 (24—1551)
TO-TO5	38	1	0.65 (0.04)	0.63 (0.03)	-0.03 (0.04)	3.80 (0.29)		
VK	96	96	0.61 (0.04)	0.65 (0.04)	0.07 (0.04)	5.19 (0.39)		
VK-PA	28	28	0.60 (0.05)	0.67 (0.04)	0.11 (0.04)	4.17 (0.28)	134 (79—390)	52 (31—97)

VK-PB	26	26	0.63 (0.04)	0.65 (0.04)	0.02 (0.04)	4.07 (0.28)	445 (121—∞)	50 (31—98)
VK-PE	18	18	0.58 (0.05)	0.61 (0.04)	0.03 (0.05)	3.65 (0.24)	25 (18—38)	36 (19—73)
VK-PF	19	19	0.59 (0.05)	0.60 (0.04)	0.00 (0.05)	3.52 (0.22)	20 (16—26)	25 (13—52)
WR	61	35	0.60 (0.05)	0.58 (0.04)	-0.04 (0.05)	4.11 (0.38)		
WR-WR2	13	13	0.57 (0.05)	0.56 (0.04)	-0.04 (0.06)	3.10 (0.24)	44 (22—352)	31 (16—82)
WR-WR3	48	22	0.61 (0.05)	0.57 (0.04)	-0.07 (0.05)	3.24 (0.24)	56 (39—92)	42 (23—89)
WT	172	145	0.53 (0.05)	0.50 (0.05)	-0.07 (0.05)	3.34 (0.32)		
WT-WT1	41	41	0.52 (0.06)	0.49 (0.05)	-0.05 (0.06)	2.81 (0.24)	117 (72—275)	61 (36—128)
WT-WT2	19	19	0.52 (0.05)	0.50 (0.05)	-0.05 (0.06)	2.83 (0.23)	∞ (165—∞)	57 (30—147)
WT-WT4	81	60	0.55 (0.05)	0.51 (0.05)	-0.09 (0.05)	2.91 (0.24)	84 (63—120)	59 (40—89)
WT-WT6	30	24	0.54 (0.06)	0.50 (0.05)	-0.08 (0.06)	2.82 (0.23)	77 (43—263)	32 (19—61)
ZE	43	43	0.46 (0.05)	0.44 (0.04)	-0.02 (0.08)	2.72 (0.18)		
ZE-ZE6	38	38	0.46 (0.05)	0.43 (0.04)	-0.03 (0.08)	2.41 (0.15)	45 (30—78)	28 (17—49)

SSP: spatially structured population, N: total sample size, Na: number of adults and metamorphosed juveniles, A_R: allelic richness, H_E: expected heterozygosity, H_O: observed heterozygosity, F_{IS}: inbreeding coefficient, N_e: effective population size calculated for adult samples using the linkage disequilibrium method (LD-N_e) and the sibship assignment method (SA-N_e)

Table 2 Model averaged results of the top models with ΔAICc ≤ 2 for each of the response values and buffer sizes. Coefficients are given for each covariate still included in the model

Response	Buffer radius (m)	Within-SSP-distance to the nearest pond	Within-SSP-number of lentic systems	Within SSP-surface water	Within-SSP-arable land	Within-SSP-pasture	Between-SSP-pasture	Within-SSP-trees	Between-SSP-trees	Within-SSP-grassland and shrubs	Within-SSP-roads
A_R	50	-0.001***	-0.017*	1.765***	0.225						
	100	-0.001***	-0.014***	3.196***	0.749**	0.521*		-0.093			
	250	-0.001**				0.126					
	500	-0.001***		-0.672	-1.576*						
F_{IS}	50	-0.0002*				-0.222**	0.013	-0.048			
	100	-0.00002						-0.169**	0.025		
	250	-0.00001	0.0002		-0.031			-0.352*	0.010		-0.000004
	500	-0.0001			-0.103	-0.016			0.020		
LD-N_e	50		0.658		0.557				3.756*		
	100			10.192					2.297		
	250	0.0005				0.237		2.718			
	500			-26.854					1.392		
SA-N_e	50	-0.0002	-0.021	0.398	0.0856						
	100	-0.0028*		0.344				-0.907	0.144		
	250	-0.002									-0.0002*
	500	-0.002							0.543	0.213	

SSP: spatially structured population, N_e: effective population size calculated for adult samples using the linkage disequilibrium method (LD-N_e) and the sibship assignment method (SA-N_e), significant *p*-value indicated as < 0.05 (*), < 0.01 (**), < 0.001 (***)

Figure captions

Fig. 1 Sampled spatially structured populations of great crested newt in Belgium indicated with black dots. The inset map shows part of Europe with Belgium indicated in grey. The background for Belgium is built from the Corine land cover data of 2018 (http://dataservice.eea.europa.eu) with the five major classes depicted here

Fig. 2 Bar plot of the Bayesian Analysis of Population Structure (BAPS) results (upper) and of the Discriminant Analysis of Principal Components (DAPC) results (lower) of great crested newt genotypes. The different colours represent different genetic clusters (15) with population codes given on the x-axis. Ponds that clustered separately according to the BAPS results are indicated above the plot

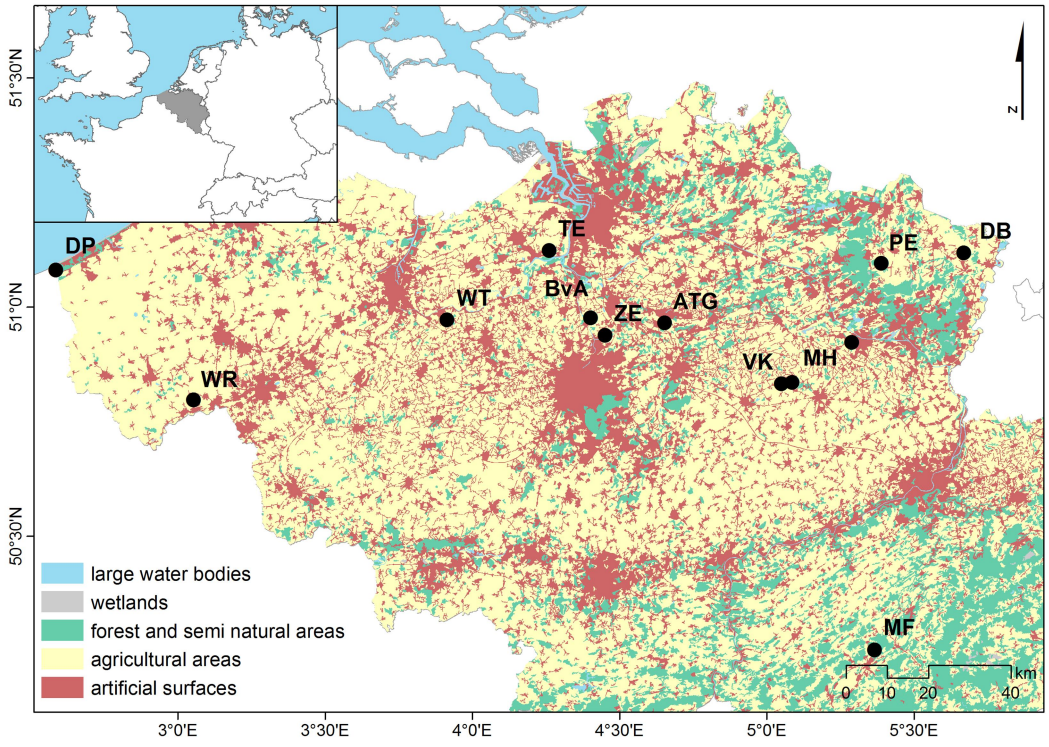
Fig. 3 Relationship between allelic richness (A_R) and each significantly contributing landscape variable at the different buffer sizes. Full model averaged results for within-SSP effects are shown for buffer radius 50 m (a), 100 m (b), 250 m (c) and 500 m (d) with fitted regression lines and 95% confidence interval (grey shaded area)

Fig. 4 Relationship between inbreeding coefficient (F_{IS}) and each significantly contributing landscape variable at the different buffer sizes. Full model averaged results for within-SSP effects are shown for buffer radius 50 m (a), 100 m (b) and 250 m (c) with fitted regression lines and 95% confidence interval (grey shaded area)

Fig. 5 Relationship between log transformed estimates of effective population size based on the sibship assignment approach (SA-N_e) and each significantly contributing landscape variable at the different buffer sizes. Full model averaged results for within-SSP effects are shown for buffer radius 100 m (a) and 250 m (b) with fitted regression lines and 95% confidence interval (grey shaded area)

Fig. 6 Relationship between log transformed estimates of effective population size based on the linkage disequilibrium approach (LD-N_e) and between-SSP effect of proportion of woodland within a buffer radius of 50 m with fitted regression line and 95% confidence interval (grey shaded area)

Figure 1


51°30'N
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large water bodies
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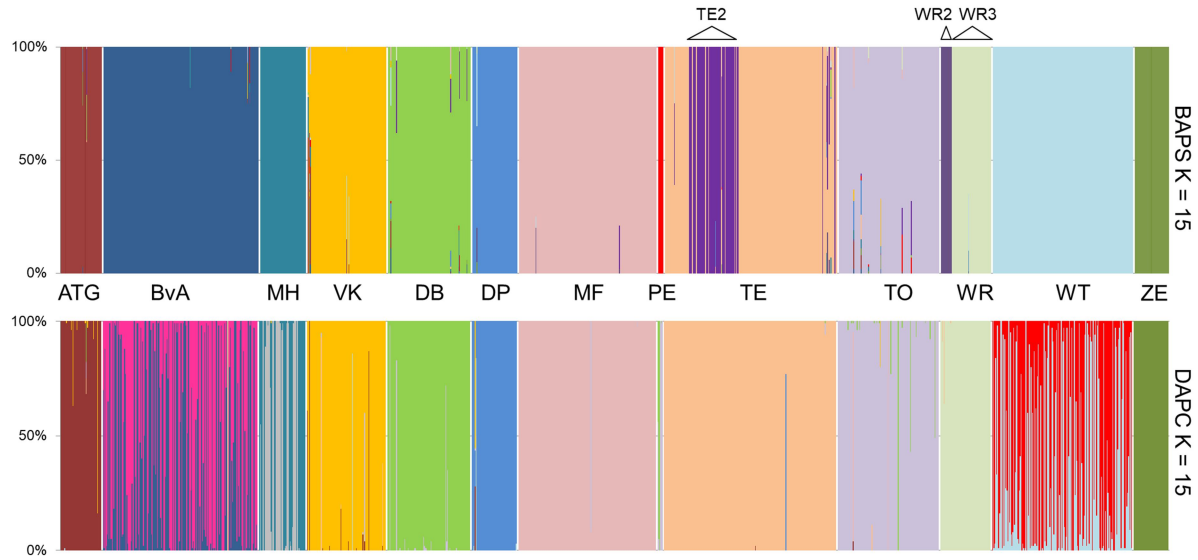
Figure 2


TE2
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100%
50%
0%
DAPC K = 15

Figure 3

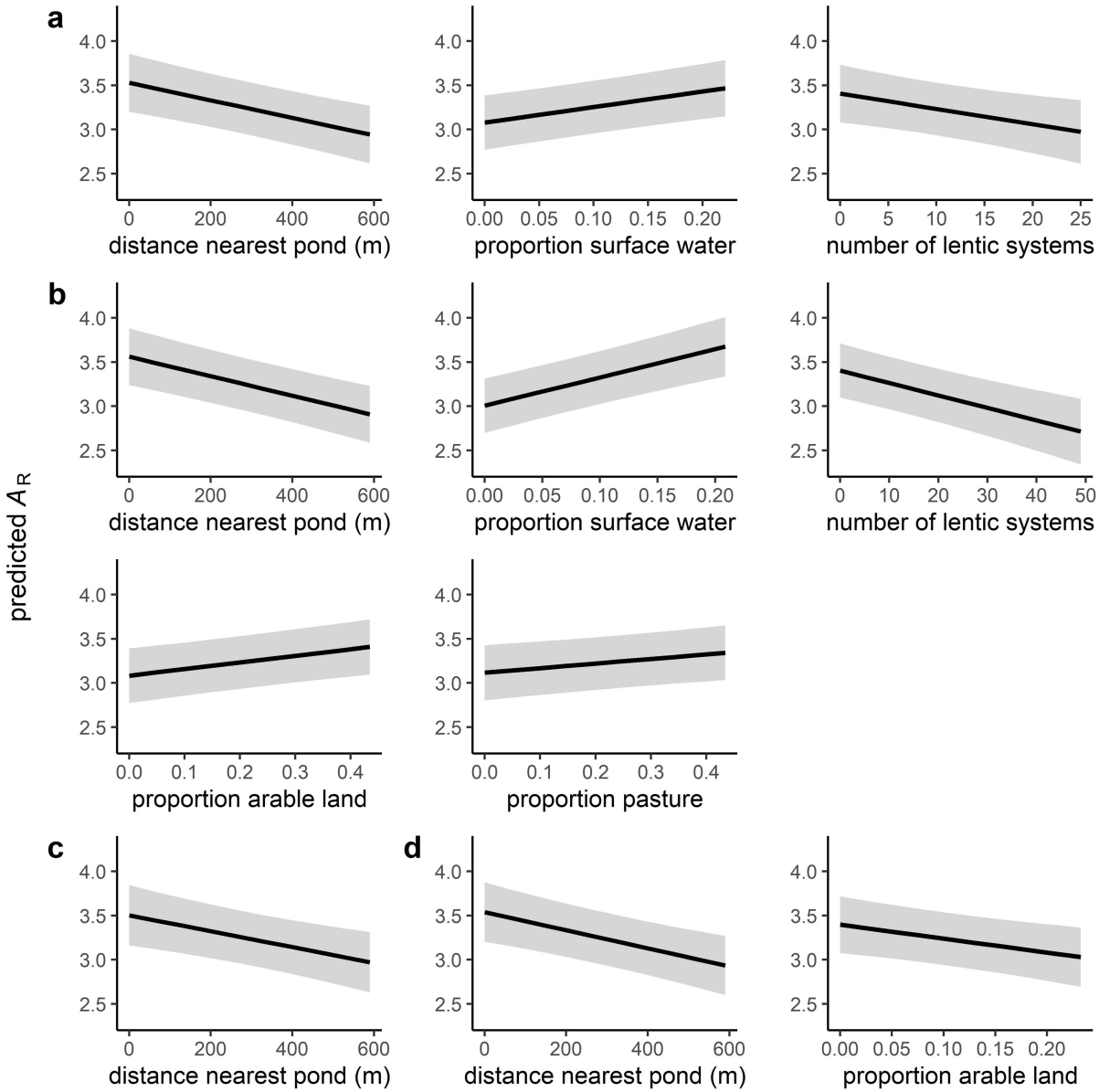
a
b
c
d
predicted A_R
distance nearest pond (m)
proportion surface water
number of lentic systems
proportion arable land
proportion pasture

Figure 4

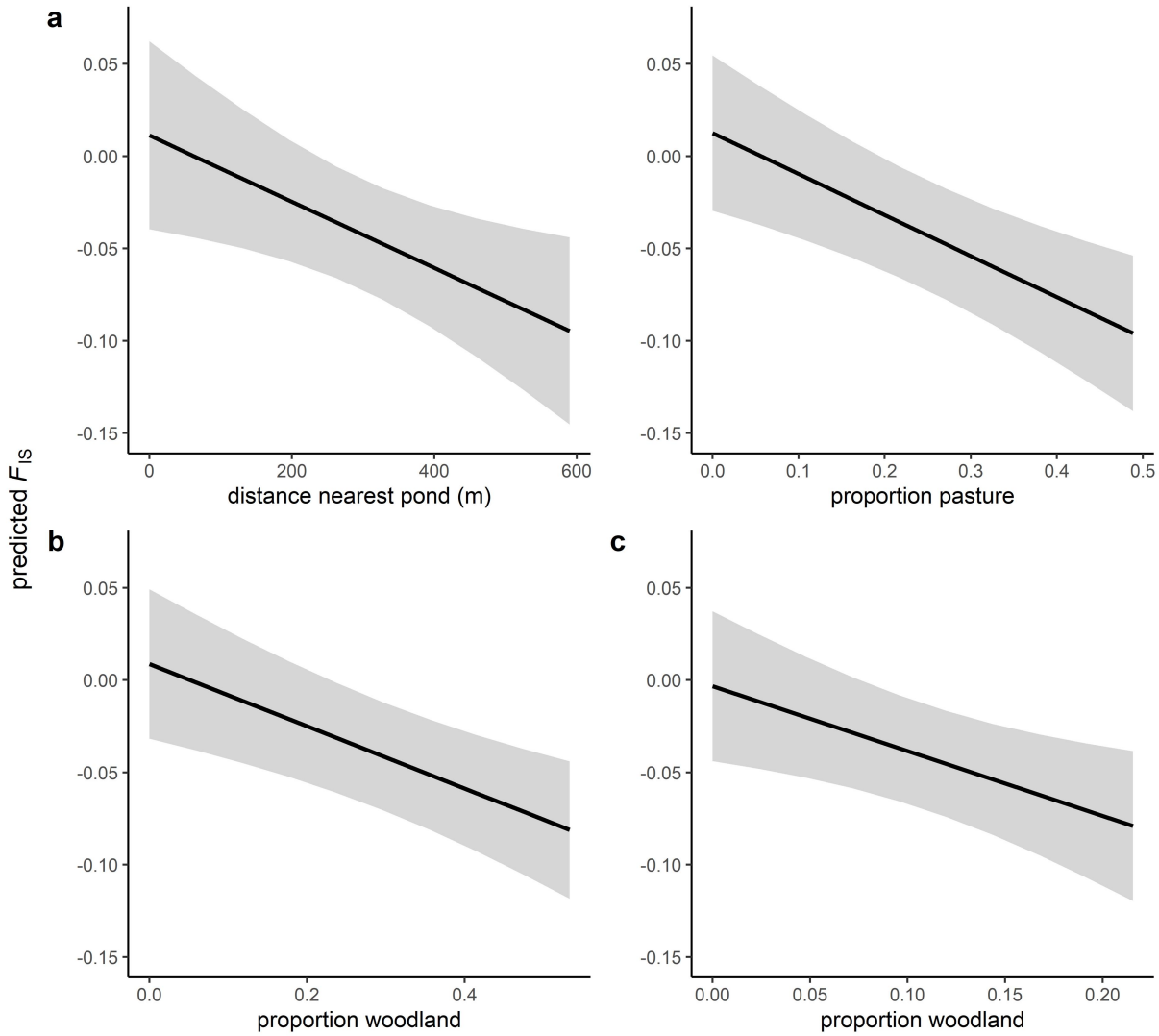
a
b
c
predicted F_{IS}
0.05
0.00
-0.05
-0.10
-0.15
0
200
400
600
distance nearest pond (m)
0.0
0.1
0.2
0.3
0.4
0.5
proportion pasture
0.2
proportion woodland
0.00
0.05
0.10
0.15
0.20

Figure 5

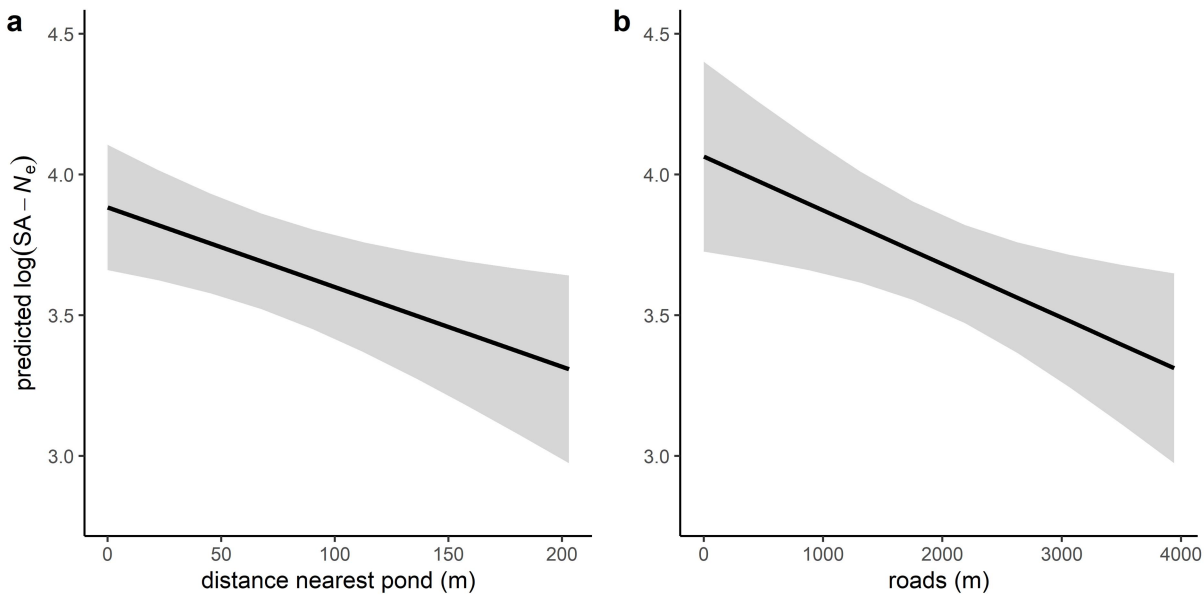
a
b
predicted log(SA – N_e)
4.5
4.0
3.5
3.0
0
50
100
150
200
distance nearest pond (m)
0
1000
2000
3000
4000
roads (m)

Figure 6