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RESEARCH ARTICLE

Spatial prioritisation for crested newt conservation in Luxembourg: Insights from population genetics and species distribution modelling

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Abstract

1. The intensification of agriculture and urbanisation has precipitated the decline of numerous species, including the northern crested newt (*Triturus cristatus*). To mitigate the impacts of habitat degradation and loss, restoration initiatives depend on evidence-based decision-making to identify priority sites for conservation efforts. Here, we employed a multidisciplinary approach, drawing on population genetics, species distribution modelling (SDMs) and landscape genetics to develop a spatial prioritisation framework for the creation and/or restoration of crested newt breeding ponds in Luxembourg.
2. For the genetic analyses, more than 1000 crested newts from 98 ponds across Luxembourg were genotyped at 15 microsatellite loci to assess genetic diversity and spatial clustering. The relationship between newt presence and environmental variables was evaluated using SDMs. Priority restoration areas were identified with PRIORITZR, integrating genetic diversity, habitat suitability and connectivity.
3. The population genetic structure was characterised by spatial clustering, which followed an isolation-by-distance pattern. Five sites were identified as significantly differentiated from the remaining populations, warranting targeted conservation efforts.
4. Standing water bodies and proximity to the nearest occupied pond were the most important predictors of crested newt presence. The highest gain in habitat suitability through the creation of new ponds was predicted to occur in southwest Luxembourg.
5. Based on the country-wide predicted occurrence of crested newts and gains in habitat suitability, areas of highest restoration potential were identified in close proximity to existing crested newt populations. The produced maps of priority restoration areas should inform future conservation efforts of the crested newt in Luxembourg.

6. While the importance of preserving genetic diversity is widely recognised, the theory-practice gap frequently precludes the incorporation of genetic aspects into in situ conservation measures or management policies. This case study demonstrates the integration of multidisciplinary analyses within an evidence-based framework to guide species conservation efforts.

KEYWORDS
amphibian, conservation, genetic diversity, habitat suitability, population structure, restoration, spatial prioritisation, *Triturus cristatus*

1 | INTRODUCTION

Species and natural habitats face an increasing number of threats as a result of land-use changes, fragmentation, introduction of invasive species and climate change (Kennedy et al., 2019; Sala et al., 2000). Conservation practitioners and land-use planners assume the challenging task of developing cost-efficient strategies to ensure that threatened species and habitats are maintained at, or restored to, a favourable conservation status. Habitat restoration projects aim to counter some of the above threats through the purposeful rehabilitation of areas to recreate functioning ecosystems and preserve species. An evidence-based framework can greatly improve the effectiveness of management decisions and provide guidance on spatial prioritisation, that is, the identification of priority areas for conservation actions (Sinclair et al., 2018).

Practitioners may draw on studies of species distribution modelling (SDMs), movement ecology, population and landscape genetics to design restoration projects (e.g. Angelieri et al., 2016; Raeymaekers et al., 2009; Wilson et al., 2011). SDMs aim to quantify the relationship between species occurrence and environmental or physical landscape features (Guisan & Thuiller, 2005; Guisan & Zimmermann, 2000). These models have been applied in conservation to reconstruct past distributions of species, to identify contemporary suitable habitat and to project species distribution into the future under different climate or restoration scenarios (Angelieri et al., 2016; Hijmans & Graham, 2006; Svenning et al., 2011). Studies on movement ecology elucidate temporal and spatial trends in species dispersal; information that is crucial to define the species' dispersal capacities to recolonise restored habitats (Barton et al., 2015; Brederveld et al., 2011). Population genetic studies characterise the amount of genetic connectivity among persisting assemblages, thereby providing information on population isolation, metapopulation compositions, or source-sink dynamics. Inferences drawn from population genetics may, therefore, help managers to identify populations at the highest risk of extinction or to assess connectivity among restored habitats (Frankham, 2005; Lande & Barrowclough, 1987; Proft et al., 2018). In landscape genetics, the effects of landscape structures on genetic connectivity are quantified to identify features that may hinder or facilitate gene flow among populations (Manel & Holderegger, 2013). Knowledge gained from landscape genetics has been useful in the design of wildlife corridors (Epps et al., 2007) and in assessing the effects of landscape fragmentation (Van Strien et al., 2014; Williams et al., 2003). The greatest power in conservation can be achieved by combining the results of complementary studies to design species management plans (McCluskey et al., 2022; O'Brien et al., 2021; Proft et al., 2018).

Habitat restoration actions are particularly important for the conservation of amphibians that have been undergoing global declines linked to the interplay of multiple stressors, including habitat loss, climate change and diseases (Moor et al., 2022). Here, we draw on the multidisciplinary field of landscape ecology to provide an evidence-based framework for the conservation of the northern crested newt (*Triturus cristatus*) in Luxembourg, where this species is legally protected. The northern crested newt (crested newt hereafter) is currently facing local declines throughout its range, rendering it one of the rarest newt species in western and central Europe (Denoël, 2012; Grillas et al., 2018). The species is listed in Appendix II of the Bern Convention, and, in the European Union, on Annexes II and IV of the Habitats and Species Directive. Destructions of both aquatic and terrestrial habitats through the intensification of agriculture and land use have been identified as key threats to the crested newt (Edgar & Bird, 2006). The countryside in Luxembourg is marked by intensive agriculture and the country has one of the highest levels of habitat fragmentation and urban sprawl in Europe (European Environment Agency, 2016; European Environment Agency et al., 2011). By the 1980s around two-thirds of crested newt populations in known breeding ponds had disappeared and an action plan was implemented in 1993 to restore and create water bodies for amphibians (Gerend, 1994). More than 500 ponds have since been restored or created in Luxembourg, with a focus on the southwestern region which holds most crested newt occurrences (Glesener et al., 2022). Compared to the period of 1980–1999, the occurrence of crested newts in southwestern Luxembourg has increased from 4.1% to 7.6% in 2010–2021 (in terms of presence in 987 1 × 1 km grid cells; Glesener et al., 2022). The population expansion was aided in part by the availability of newly created ponds, of which 14.1% have thus far been colonised (Glesener et al., 2022). However, an evidence-based framework could significantly improve the rate of successful colonisations by identifying areas of highest colonisation probability that should be prioritised in habitat restoration and renaturation efforts (O'Brien et al., 2021).

The aim of the present study was to identify the best locations for the restoration and creation of crested newt breeding ponds in Luxembourg that would support genetically isolated crested newt populations and consider the species' dispersal capacities. We employed a multidisciplinary approach, drawing on population genetics, SDMs and landscape genetics to develop an evidence-based spatial prioritisation strategy and report the results of analyses characterising crested newt aggregations in terms of genetic differentiation, diversity and environmental associations. We performed the first nationwide genetic assessment of the crested newt in Luxembourg to assess the extent of population fragmentation. This evaluation served to identify the most isolated populations that may face an increased extinction risk and may, therefore, benefit most from conservation actions that aim to increase local population sizes or improve connectivity within the metapopulation. SDMs were employed to quantify the gain in habitat suitability by the creation of new breeding ponds. For the purpose of spatial conservation prioritisation, these results were subsequently integrated with known aspects of the species' movement ecology and results from an associated landscape genetic analysis (Schleimer et al., 2023) to ensure low landscape resistance and high connectivity within pondscapes. The integrated results yielded a map with priority areas for the restoration and creation of crested newt habitat that can be easily translated to on-the-ground conservation actions.

2 | MATERIALS AND METHODS

2.1 | Study species

The northern crested newt is a pond-breeding amphibian that disperses seasonally between aquatic and terrestrial habitats. In spring, subadult and adult crested newts seek out breeding ponds, where they may stay for up to 5 months (Thiesmeier et al., 2009). Breeding ponds are generally large standing water bodies characterised by high solar radiation, extensive vegetation and absence of fish (Denoël et al., 2013; Hartel et al., 2010). Two behavioural phenotypes have been described in relation to intra- and interseasonal site fidelity and dispersal (Denoël et al., 2018). Some individuals show strong fidelity to their breeding ponds and, during the terrestrial phase, most animals stay within close proximity of their breeding pond (<100 m; Jarvis, 2016; Jehle, 2000), while others disperse among nearby (some hundred meters) suitable breeding ponds within and between seasons (Denoël et al., 2018). Terrestrial habitats include rank grass and deciduous forests where the newts seek refuge under rotting tree logs, rocks, or in burrows of rodents (Thiesmeier et al., 2009). Long-distance dispersal, by both males and females, is rare but plays an important role in the colonisation of new habitats (Jarvis, 2016; Pittman et al., 2014). The maximum reported dispersal distances by adult crested newts are 1290 m (Kupfer, 1998) and 1610 m (Haubrock & Altrichter, 2016), but long-distance dispersal may be more common in juveniles with a maximum recorded distance of 860 m from natal ponds within 1 year of metamorphosis (Jarvis, 2016; Kupfer & Kneitz, 2000). Based on shifts in a genetic hybrid zone, Arntzen and Wallis (1991) estimated a dispersal distance of 1 km per year.

In Luxembourg, the species occurs exclusively in the southern part of the country, the Gutland, except for a single isolated population at the 'Trëtterbaach' in the north of the Ösling (Proess, 2016; Figure 1). The first record of the northern animals dates to 1972 and they were included on the species distribution maps by Risch (1973) and Parent (1979) without any known nearby source populations. In the 1984 atlas by Parent (1984), three additional occurrences in the neighbouring Germany were noted but whether they had already been present in the 1970s and could have served as source populations remains unclear. According to anecdotal stories and given its remote location, it has been suggested that they were introduced by humans from southern Luxembourgish populations. There is at least one known translocation of crested newts from the area of Redange to the ca. 30 km distant Dudelange based on anecdotal information.

2.2 | Genetic sample collection and processing

Tissue samples for population genetic analyses were collected from crested newts in spring 2019 and 2020. Three to four Laar newt traps per site were set in 98 ponds across Luxembourg and their contents were checked the following day. The Laar trap is a rectangular swim-trap, kept afloat with two pontoons and two nylon funnel openings to facilitate the capture of amphibians (https://www.laartech.biz/LTCGroup-int/html/newt_trap.html). Non-lethal tail clips, that is, 3–5 mm of the tail tip, were collected from captured newts and stored in 96% ethanol. All fieldwork was performed under permits issued by the Ministry of the Environment, Climate and Biodiversity, Luxembourg (92,671 CD/gp, 95,227 & 95,827 CD/ne).

DNA extractions from tissue samples were performed with an ammonium acetate salting-out procedure (Miller et al., 1988). Samples were genotyped at 15 microsatellite loci (Drechsler et al., 2013; Krupa et al., 2002; Sotiropoulos et al., 2008) using three multiplex reactions as described in detail by Schleimer et al. (2023).

2.3 | Population genetic analysis

Departures from Hardy–Weinberg proportions within local subpopulations (ponds with $N \geq 14$ individuals) were estimated as F_{IS} using the R package HIERFSTAT (v. 0.04–22; Goudet, 2005). Observed heterozygosity (H_O), expected heterozygosity (H_E) and rarefied allelic richness (A_R) were estimated with the DIVERSITY R package (v.1.9.90; Keenan et al., 2013) for ponds with sample sizes $N \geq 14$ individuals. The minimum number of 14 individuals was chosen based on resampling analysis with a subset of the data as reported in Schleimer et al. (2023). Population-specific F_{ST} (mode) with 95% highest posterior density interval was estimated

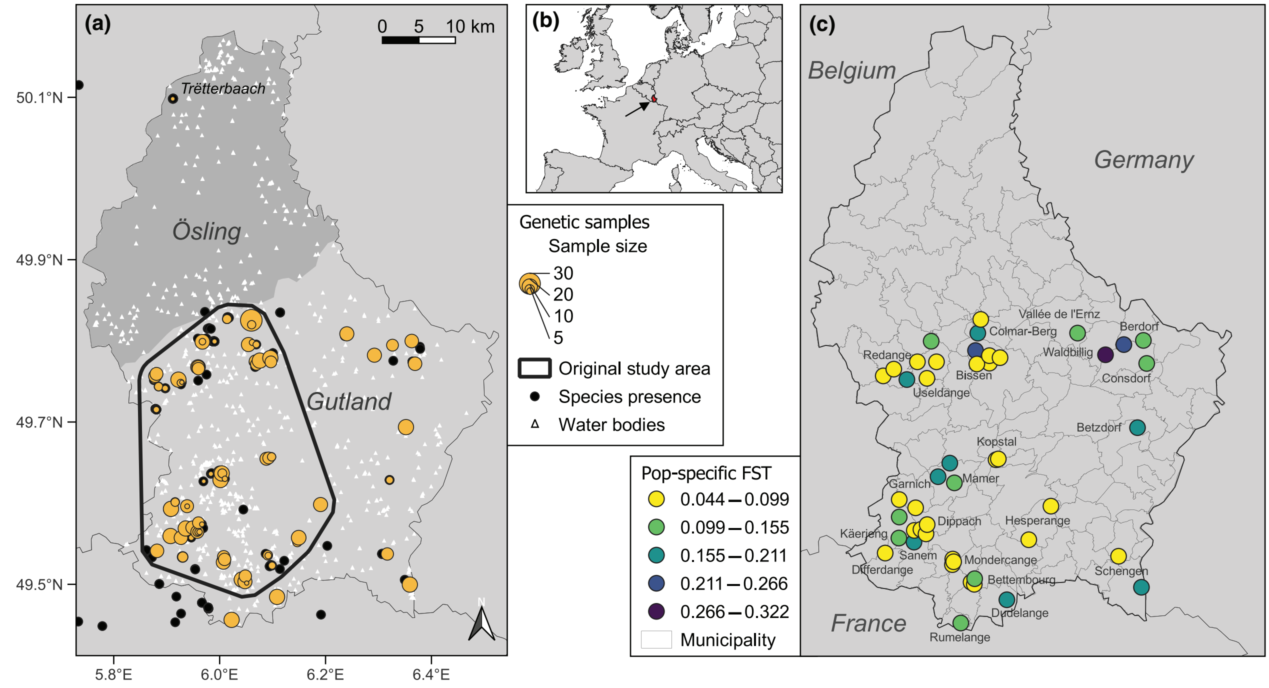

FIGURE 1 Distribution of known occurrences of the northern crested newt (*Triturus cristatus*) and genetic sample locations (a). The original study area refers to the spatial extent of the landscape genetic study by Schleimer et al. (2023). The arrow shows the location of Luxembourg in Europe (b). The population-specific estimates of F_{ST} for sample locations with $N \geq 14$ are colour-coded in panel (c).

in GESTE (v.2; Gaggiotti & Foll, 2010). Pairwise genetic differentiation was estimated as Weir and Cockerham's (1984) F_{ST} with HIERFSTAT.

The number of distinct genetic clusters K with the highest support was estimated using the Bayesian clustering approaches implemented in STRUCTURE (v.2.3.4; Pritchard et al., 2000) and BAPS (v.6; Corander et al., 2008). For non-spatial clustering in STRUCTURE, an admixture model with correlated allele frequencies was assumed. The population-specific ancestry prior and $\alpha = 1/K$ were applied as recommended by Wang (2017). Markov-Chain Monte-Carlo (MCMC) iterations consisted of a burn-in period of 300,000, followed by 1,000,000 MCMC iterations for each K, ranging from one to 20. For each value of K, 10 replicates were performed to assess consistency among runs. The most likely number of plausible clusters was inferred from the estimated posterior probability for the data $LnP(D|K)$. The spatially explicit model of BAPS was employed to conduct spatial clustering of individuals. The most likely number of clusters (K) in terms of highest log marginal likelihood, was inferred from 100 replicate runs with a maximum $K = 40$.

The presence of an isolation-by-distance pattern was assessed using genetic distances between all individuals and between populations with $N \geq 14$ individuals. Pairwise estimates of Loiselle's kinship coefficient (Loiselle et al., 1995) and linearised F_{ST} (Weir & Cockerham, 1984), estimated as $F_{ST}/(1 - F_{ST})$, were regressed against the natural logarithm of geographic distances in SPAGEDI (v.1.5d; Hardy & Vekemans, 2002) for the individual-based and population-based approach, respectively. The probability of the regression slope was estimated from 10,000 permutations of locations. Standard errors were estimated from jackknifing over loci.

To test the hypothesis that the northernmost crested newt population in Luxembourg originated from an introduction from the south, an assignment/exclusion test was carried out using GENECLASS2 (v.2.0.h; Piry et al., 2004). The approach does not assume that all potential source populations were sampled. The probability of the suspect individual belonging to one of the predefined clusters was estimated using the Bayesian method by Rannala and Mountain (1997) and Paetkau et al.'s (2004) modified Monte-Carlo resampling with 10,000 simulated individuals and $p < 0.01$ threshold for excluding populations. The 20 clusters inferred from BAPS were employed as reference populations, with an additional run with a higher hierarchical level at $K = 6$ clusters based on STRUCTURE.

2.4 | Habitat suitability

2.4.1 | Species data

Occurrence data of crested newts in Luxembourg were extracted from the RECORDER national database (Musée national d'histoire naturelle, MNHN) for the period of 2010–2020. Sightings were supplemented with the occurrence data from the landscape genetics field campaign of 2019–2020. Detection records were subsequently spatially aggregated within 200-meter resolution grid cells using the Luxembourg 1930/Gauss (LUREF) coordinate reference system.

2.4.2 | Environmental data

Environmental variables were selected based on known ecological factors influencing amphibian habitat use. Eighty-two predictor variables were considered, in relation to climate, elevation, land cover, rivers and geology (Supporting Information, Table S1). Country-wide environmental data, available at various resolutions, were resampled to 200-m grid cells using the same LUREF coordinate system as for the species records.

Collinearity among predictor variables was assessed using routine select07 (Dormann et al., 2013), with pairwise Spearman's rank correlation coefficients based on species presence and a random selection of 10,000,200-m grid cells. In the case of high collinearity (Spearman's $r > 0.7$), a univariate Generalised Linear Model was used using occurrence records as a response, and the predictor variable with the highest explanatory power was retained.

2.4.3 | Species distribution modelling

The relationship between crested newt detections and environmental variables was estimated using the maximum entropy algorithm as implemented in MaxEnt (v. 3.3.3k; Phillips et al., 2004, 2006). The MaxEnt approach was chosen because the detection data extracted from RECORDER consisted of presence-only data. Pseudo-absences were generated from a random selection of 10,000 background 200-m grid cells. In the first stage, models were fitted with all non-correlated environmental variables and their quadratic terms, using cross-validation with a 10-fold partitioning procedure to define the calibration datasets and to statistically evaluate their performance. Individual models were then averaged to produce a full single model. In the second stage, models were built using only explanatory variables that were considered to significantly contribute to the fit of the first full averaged model (permutation importance and contribution >1 in the full model). Model performance was assessed using the Area Under the Curve (AUC) of the receiver operating characteristic (ROC) curve and the omission rate statistics. Models were considered useful if AUC values were >0.80 (Swets, 1988). The average model outcomes were used to generate a nationwide surface of relative habitat suitability (values from 0 to 1) for the crested newt in Luxembourg.

2.4.4 | Habitat suitability projection

To identify areas which would benefit most from the creation of standing water bodies, in terms of improvement of habitat suitability, a second predictive map was produced using the same reduced set of environmental variables (see 2.4.3) but assuming an increase in pond coverage. The environmental predictor ‘Standing water’ was thus modified in all grid cells to 132 m^2 when the original value was below this chosen threshold. This threshold corresponded to the average coverage size of ponds in grid cells where crested newt occurrence had been confirmed in Luxembourg. A new predictive surface of habitat suitability was generated with this adapted environmental variable. Areas with the highest gain in habitat suitability were identified by subtracting the original habitat suitability indices from these modified ones (ΔHS).

2.5 | Landscape resistance extrapolation

A landscape genetics study for crested newts was previously conducted over an area of 600 km^2 in southwestern Luxembourg (Schleimer et al., 2023). Briefly, resistance surfaces were optimised using genetic algorithms in ResistanceGA R package (v.4.1.0.45; Peterman, 2018). ResistanceGA fits linear mixed-effect models with a maximum likelihood population effects parameterisation, using pairwise genetic distance as response and pairwise effective cost distance as predictor variables (Clarke et al., 2002; Winiarski et al., 2020). Based on simulations, Schleimer et al. identified Jost's D (Jost, 2008), F_{ST} (Weir & Cockerham, 1984) and Principal Component Axes (PCA) 1–45 as best performing population-pairwise genetic distances for the given dataset. These optimised resistance surfaces and derived current maps generated in CIRCUITSCAPE were employed here as explanatory variables to understand the importance of landscape resistance with respect to other variables. A nationwide landscape resistance surface for crested newts was generated by extrapolation of the best-performing surface in the model limited to the original study area (see below). The optimised parameters for transformation, shape and magnitude were employed with the *Combine_Surfaces*() function to generate an extrapolated resistance surface. The correlation between the original and extrapolated resistance surface within the original study area of Schleimer et al. (2023) was estimated as Pearson's correlation coefficient r in *layerStats*() in the RASTER R package (v.3.6–14; Hijmans, 2023).

2.6 | Generalised additive modelling

2.6.1 | Model description

Generalised additive models (GAMs; Hastie & Tibshirani, 1990; Wood, 2017) were fitted to understand the relative contribution of landscape resistance, habitat suitability and the distance to the closest occupied ponds in explaining crested newt occurrence. Response and explanatory data were summarised per 200 × 200 m grid cell that included at least one freshwater body. The successful or unsuccessful detections of crested newts per grid cell from 2010 to 2020 formed the response variable and was modelled with a binomial error distribution and logit link function in the *mgcv* R package (v. 1.8–41; Wood, 2011). Restricted maximum likelihood (REML) was used for smoothing parameter estimation (Marra & Wood, 2011). The gamma term, which acts as an additional penalty, was set to 1.4 to reduce overfitting (Kim & Gu, 2004; Wood, 2006).

2.6.2 | GAM within original study area

In the first stage, model fitting was limited to data within Schleimer et al.'s original study area to test the performance of different surfaces derived from the landscape genetic analysis from that study. Four candidate explanatory variables were considered; (1) the habitat suitability index, (2) the distance to the nearest grid cell with positive crested newt detections (other than itself), (3) the optimised landscape resistance and (4) the landscape connectivity as inferred from the current maps generated in CIRCUITSCAPE (McRae, 2006) in Schleimer et al. (2023). Due to high collinearity among resistance and connectivity surfaces (see Results), three separate models were fitted, each with one resistance and connectivity surface derived from one of the three tested genetic distances, that is, Jost's *D*, F_{ST} and PCA 1–45. Model performance was evaluated in terms of percentage deviance explained and Akaike information criterion (AIC; Akaike, 1973; Burnham & Anderson, 2002).

2.6.3 | GAM within the entire country

In the second stage, a GAM was fitted to the data available for the entire country using three candidate explanatory variables: (1) the habitat suitability index, (2) the distance to the nearest grid cell with positive crested newt detections (other than itself), (3) the extrapolated landscape resistance surface for the best-performing genetic distance in the study area model. Since the nearest pond with confirmed crested newt presence could be located outside Luxembourg, sightings were extracted for the 2010–2020 period from the Global Biodiversity Information Facility for Germany and France and from Observations.be for Belgium.

2.6.4 | Model selection

For both the study area and nationwide GAMs, covariate selection was carried out using shrinkage spline smooths, which penalises the null space of the smooth function and reduces the degrees of freedom of all unsupported covariates to zero in a single step (Marra & Wood, 2011). The relative importance of retained covariates was estimated with the R function *varImpBiomod* (Thuiller et al., 2009). Randomised quantile residuals were plotted to check for heteroscedasticity using rqgam_check in the DSM R package (v. 2.3.3; Miller et al., 2022). Spatial autocorrelation of model residuals was further assessed using a variogram (Zuur et al., 2009).

The final fitted GAM was used to create a predictive nationwide surface of crested newt occurrence. To assess the potential biases due to false absences of crested newts, we created 100 datasets where a random selection of 5% of the non-detection grid cells was relabelled as presences. After updating the distance to the nearest occupied pond, the nationwide GAM was refitted using the same explanatory variables and model selection procedures as described above. The percentage of deviance explained significant covariates (*p*-values) and the covariate importance were recorded for each of the 100 simulated GAMs. The loop was repeated with a false absence rate of 10%.

2.7 | Spatial prioritisation

The spatial prioritisation of crested newt habitat management was based on a set of five criteria, informed by the results of the population genetics, landscape resistance, habitat suitability analyses and the scientific literature. The criteria consisted of (1) high habitat suitability, (2) close proximity to a pond with known crested newts occurrence, (3) high connectivity among identified areas, (4) less than 25% impervious surfaces, (5) a distance of at least 1 km to the nearest motorway (Hamer et al., 2021). This prioritisation problem was solved using the PRIORITIZR R package (v.8.0.0; Hanson et al., 2023) with a relative target of 30%, in line with the national goal of protecting 30% of the territory (MECDD, 2023). A raster layer with soil impermeability was obtained from data.public.lu and geospatial vector data for motorways were obtained from OpenStreetMap and modified to exclude tunnel segments. The planning units consisted of a countrywide single-layer raster, at 200 × 200 m resolution, with an equal cost of 1 assigned to each cell. The feature layer consisted of the predicted occurrence of crested newts, as derived from the final GAM, thus incorporating the effects of habitat suitability and pond proximity. A linear constraint was added to the prioritisation problem based on the distribution of areas which showed the highest gain in habitat suitability when standing water bodies were added. The highest gain was defined in terms of areas where the difference between the original and projected habitat suitability fell within the upper quartile of ΔHS ($\Delta HS > 0.05$). A constraint threshold of 700 was chosen based on the conservation target to create 700 ponds for amphibians by 2030 (MECDD, 2023). Additionally, a connectivity penalty was employed based on the inverted raster of the extrapolated resistance surface, with the penalty level set to 1.2×10^{-6}. The threshold was chosen as a trade-off between high connectivity and low cost.

3 | RESULTS

3.1 | Population genetics

Genotyping data were generated at an average of 14.8 microsatellite loci (minimum 13 loci) in 1068 unique individuals. Samples were obtained from 98 locations across Luxembourg (Figure 1), with an average of 10.9 samples per surveyed pond (minimum 1, maximum 24). Summary statistics were estimated for the 47 locations with $N \geq 14$ individuals (Table 1). Average expected and observed heterozygosities were estimated at 0.47 and 0.50, respectively. None of the inbreeding coefficients significantly deviated from 0, except for one location in Rumelange ($F_{IS} = -0.078$, 95% confidence interval −0.1747, −0.0068). The population-specific F_{ST} ranged from

TABLE 1 Summary statistics for 47 ponds with $N \geq 14$ samples ordered from north to south.

Pop	Municipality	Lat [°N]	Lon [°E]	*N*	Ar	He	Ho	Fis	CI	Fst	95% HPDI
Pond1	Colmar-Berg	49.8261	6.0588	24	3.62	0.47	0.51	−0.063	[−0.122–0.013]	0.091	[0.056–0.127]
Pond2	Vallée de l'Ernz	49.8095	6.2407	18	3.39	0.47	0.5	−0.019	[−0.150–0.094]	0.128	[0.071–0.173]
Pond3	Berdorf	49.8005	6.3643	17	3.57	0.45	0.5	−0.051	[−0.168–0.032]	0.104	[0.064–0.147]
Pond4	Useldange	49.7995	5.9658	19	3.25	0.49	0.5	−0.029	[−0.089–0.066]	0.139	[0.093–0.198]
Pond5	Bissen	49.7962	6.0534	18	3.2	0.43	0.41	0.044	[−0.028–0.163]	0.205	[0.141–0.273]
Pond6	Bissen	49.7959	6.0533	16	2.97	0.43	0.48	−0.061	[−0.201–0.050]	0.235	[0.163–0.317]
Pond7	Consdorf	49.7954	6.3276	14	2.76	0.37	0.4	−0.050	[−0.156–0.042]	0.218	[0.141–0.307]
Pond8	Waldbillig	49.7831	6.2932	18	2.43	0.36	0.39	−0.059	[−0.179–0.075]	0.322	[0.226–0.431]
Pond9	Bissen	49.7796	6.0952	20	3.52	0.46	0.49	−0.018	[−0.103–0.063]	0.085	[0.052–0.130]
Pond10	Bissen	49.7765	6.0752	20	3.77	0.5	0.54	−0.070	[−0.150–0.040]	0.074	[0.042–0.107]
Pond11	Bissen	49.7757	6.0667	15	3.5	0.45	0.46	−0.002	[−0.085–0.119]	0.097	[0.056–0.146]
Pond12	Consdorf	49.7725	6.3705	17	3.35	0.43	0.4	0.052	[−0.026–0.190]	0.123	[0.077–0.178]
Pond13	Bissen	49.7719	6.0688	14	3.54	0.47	0.5	−0.017	[−0.095–0.065]	0.086	[0.046–0.126]
Pond14	Useldange	49.7684	5.9576	19	4.09	0.53	0.53	0.000	[−0.082–0.115]	0.072	[0.046–0.106]
Pond15	Useldange	49.7684	5.9581	16	3.9	0.51	0.56	−0.079	[−0.157–0.020]	0.075	[0.045–0.109]
Pond16	Useldange	49.7664	5.9584	15	4.17	0.49	0.53	−0.038	[−0.111–0.025]	0.066	[0.039–0.099]
Pond17	Redange	49.7598	5.8786	16	3.88	0.48	0.5	−0.017	[−0.104–0.080]	0.066	[0.035–0.098]
Pond18	Redange	49.7571	5.8761	16	4.17	0.49	0.51	−0.003	[−0.059–0.061]	0.091	[0.060–0.123]
Pond19	Redange	49.7528	5.9204	20	3.22	0.43	0.45	−0.010	[−0.092–0.065]	0.168	[0.117–0.233]
Pond20	Betzdorf	49.6944	6.3529	20	3.24	0.47	0.49	−0.023	[−0.110–0.097]	0.156	[0.101–0.213]
Pond21	Kopstal	49.6565	6.0921	14	4.06	0.53	0.53	0.045	[−0.090–0.153]	0.088	[0.052–0.123]
Pond22	Kopstal	49.6555	6.0879	16	4.06	0.53	0.56	−0.050	[−0.164–0.092]	0.067	[0.039–0.098]
Pond23	Mamer	49.6385	5.9996	16	3.62	0.48	0.5	0.007	[−0.069–0.096]	0.124	[0.085–0.176]
Pond24	Mamer	49.6374	6.0031	18	3.2	0.46	0.48	−0.019	[−0.118–0.094]	0.157	[0.101–0.213]
Pond25	Mamer	49.6293	6.0008	20	3.1	0.46	0.45	0.061	[−0.026–0.133]	0.201	[0.130–0.271]
Pond26	Hesperange	49.5992	6.1906	18	3.95	0.48	0.52	−0.055	[−0.112–0.019]	0.079	[0.051–0.113]
Pond27	Garnich	49.5969	5.9376	16	4.28	0.55	0.6	−0.060	[−0.151–0.008]	0.057	[0.031–0.082]
Pond28	Kaerjeng	49.5935	5.9071	19	3.91	0.51	0.54	−0.050	[−0.117–0.042]	0.079	[0.048–0.113]
Pond29	Kaerjeng	49.5934	5.9067	19	3.55	0.45	0.49	−0.039	[−0.114–0.011]	0.110	[0.070–0.157]
Pond30	Dippach	49.5766	5.9592	14	4.05	0.48	0.5	−0.012	[−0.099–0.117]	0.063	[0.037–0.098]
Pond31	Dippach	49.5707	5.9476	16	3.91	0.51	0.54	0.009	[−0.083–0.047]	0.073	[0.043–0.107]
Pond32	Kaerjeng	49.5695	5.9352	20	4.04	0.5	0.54	−0.049	[−0.134–0.025]	0.064	[0.040–0.093]

(Continues)

TABLE 1 (Continued)

Pop	Municipality	Lat [°N]	Lon [°E]	N	Ar	He	Ho	Fis	CI	Fst	95% HPDI
Pond33	Sanem	49.5653	5.9571	14	3.54	0.46	0.49	−0.020	[−0.114–0.079]	0.096	[0.056–0.142]
Pond34	Kaerjeng	49.5601	5.9064	20	3.28	0.46	0.47	−0.003	[−0.057–0.068]	0.153	[0.102–0.207]
Pond35	Hesperange	49.5584	6.1492	19	3.98	0.5	0.5	−0.001	[−0.091–0.117]	0.081	[0.053–0.119]
Pond36	Kaerjeng	49.5580	5.9261	17	3.14	0.44	0.46	−0.041	[−0.112–0.074]	0.189	[0.121–0.257]
Pond37	Differdange	49.5418	5.8810	17	3.63	0.45	0.47	−0.032	[−0.108–0.086]	0.082	[0.046–0.121]
Pond38	Dalheim	49.5382	6.3169	15	4.39	0.49	0.51	−0.010	[−0.117–0.103]	0.044	[0.021–0.066]
Pond39	Mondercange	49.5347	6.0072	15	4.41	0.54	0.57	−0.004	[−0.095–0.077]	0.049	[0.027–0.075]
Pond40	Mondercange	49.5315	6.0089	15	4.02	0.48	0.5	−0.020	[−0.091–0.072]	0.096	[0.056–0.131]
Pond41	Mondercange	49.5280	6.0065	16	3.82	0.51	0.54	−0.035	[−0.083–0.024]	0.087	[0.055–0.125]
Pond42	Bettembourg	49.5109	6.0482	15	3.28	0.5	0.56	−0.067	[−0.175–0.030]	0.129	[0.077–0.178]
Pond43	Bettembourg	49.5067	6.0415	20	3.87	0.53	0.52	0.038	[−0.079–0.143]	0.059	[0.031–0.090]
Pond44	Bettembourg	49.5041	6.0475	18	3.55	0.47	0.5	−0.023	[−0.067–0.033]	0.079	[0.044–0.115]
Pond45	Schengen	49.5004	6.3595	20	3.09	0.43	0.42	0.049	[−0.021–0.133]	0.201	[0.138–0.276]
Pond46	Dudelange	49.4854	6.1083	20	2.97	0.39	0.43	−0.067	[−0.141–0.001]	0.177	[0.116–0.242]
Pond47	Rumelange	49.4570	6.0224	20	3.75	0.49	0.55	−0.079	[−0.174–0.005]	0.108	[0.070–0.151]

0.044 to 0.322, with the highest levels estimated for locations in the communes of Waldbillig, Bissen, Consdorf, Schengen and Mamer (all $F_{ST} > 0.2$, Figure 1, Table 1). The average pairwise estimate of F_{ST} was estimated at 0.09, with a maximum of 0.29 observed between Waldbillig and Mamer (Supporting Information, Figure S1).

Pairwise genetic differentiation was significantly correlated to geographic distance based on both Loiselle's kinship coefficient between individuals (slope ± SE = −0.012 ± 0.001, $p < 0.001$) and linearised F_{ST} between populations (slope ± SE = 0.02, $p < 0.001$). There was no clear support for a single number of clusters, based on the Bayesian clustering approach in STRUCTURE (Supporting Information, Figure S2). The likelihood increased steadily from $K = 1$ to $K = 6$, beyond which point incremental improvements were observed when increasing K with a concomitant decrease in convergence. The highest estimated posterior probability was obtained at $K = 18$, but solutions converged poorly among runs (Supporting Information, Figure S2). The best-supported clustering solution based on Evanno et al.'s (2005) ΔK method was $K = 2$. At $K = 2$, the samples were partitioned into a northern and southern cluster (Supporting Information, Figure S3A). At the mid-hierarchical level of $K = 6$, populations around Mamer, Sanem and Bissen formed distinct clusters, with an additional split between east and west (Supporting Information, Figure S3B,D). The optimal number of spatial partitions as inferred in BAPS was estimated at 20 with a probability of 0.99. Voronoi tessellation coloured by inferred cluster membership showed fine-scale spatial clustering, with most clusters largely confined to areas of close geographic proximity, similar to the clustering solution at $K = 18$ inferred from STRUCTURE (Supporting Information, Figure S4). The known translocation event from the area of Redange to Dudelange was reflected by the mixture analysis. The sample from the northernmost population clustered with samples from Dippach/Sanem in the south.

The assignment/exclusion test in GENECLASS2 could not refute the hypothesis that the suspectedly translocated individuals in Trëtterbaach originated from the south. When using the 20 reference populations inferred from BAPS, the individual was excluded from 15 potential source populations at the $p < 0.01$ threshold. The sample had the highest probability ($p = 0.21$) of belonging to the cluster of Dippach/Sanem in accordance with the BAPS mixture analysis. With the 6 clusters of STRUCTURE as reference populations, assignment tests excluded three populations and with the highest probability of assignment ($p = 0.44$) for the southern cluster ('dark blue' cluster in Supporting Information, Figure S3D).

3.2 | Species distribution modelling

Thirty-nine candidate predictor variables were excluded prior to model fitting due to high multicollinearity. Of the remaining 43 predictor variables, 15 variables had a permutation importance and contribution >1 and were retained in the final model following 10-fold cross-validation (Table 2). The model performance was found to be good, with an AUC estimated at 0.891 for the replicate runs (standard deviation: 0.048). The environmental variable that decreased the gain the most when it was omitted was standing water, which, therefore, appeared to have the most information that was not present in the other variables. Geological substrate, temperature of the driest quarter and soil type had the next highest per cent contribution. Permutation importance was highest for the mean per cent slope variable, followed by geological substrate and standing water.

TABLE 2 Predictor variables retained in MaxEnt model.

Variable	Per cent contribution	Permutation importance
Standing water	22.5	9.9
Geological substrate	22	17.4
Mean temperature of driest quarter	8.9	4.2
Soil type	6.6	1.8
Meadows and pastures 300 m	6	1.7
Mean per cent slope	5.6	32.1
Open areas	5.5	0.8
Mean temperature of wettest quarter	4.8	5.1
Annual crops	4.6	8.5
Dense urban areas	2.8	6.9
Distance to closest larger stream	2.4	3.9
Beech and oak forests	2.3	2.3
Distance to closest river	2.2	0.9
Oligo to mesotrophic waters	1.8	1.8
Rivers, 2nd Strahler order	1.8	2.6

The relative habitat suitability as estimated by MaxEnt was highest in the northwest and southwest of the Gutland (Figure 2a; Gutland/Ösling extent is shown in Figure 1a). The Ösling had the lowest relative habitat suitability, followed by the east of the Gutland. The largest gain in habitat suitability as a result of the addition of standing water bodies was also observed in the Gutland, largely coinciding with areas of high habitat suitability (Figure 2b).

3.3 | Proximity to occupied ponds as best predictor

The three resistance surfaces derived from Jost's D, F_{ST} and PCA axes 1–45 were strongly correlated, with Pearson's $r \geq 0.9$ (Supporting Information, Figure S5). Similarly, there was high collinearity among the three conductivity surfaces derived in CIRCUITSCAPE ($r \geq 0.72$; Supporting Information, Figure S5). The GAM fitted using the resistance and conductivity surfaces based on F_{ST} performed better than the models with Jost's D and PCA axes 1–45 based on the percentage deviance explained and AIC (Table 3). In all three tested models, the conductivity variables were smoothed to 0 and effectively dropped from the models. For the best-performing model GAM-F_{ST}, the probability of crested newt detection increased linearly with habitat suitability and decreased with greater distances to the nearest occupied ponds (Supporting Information Figure S6). In terms of variable importance, the distance to the nearest occupied pond was the most influential predictor variable (63.6%), followed by habitat suitability (27.9%). The F_{ST} resistance surface contributed only 5.7% to the total percentage of deviance explained.

Based on the results of GAMs fitted to the original study area, the GAM for the whole country was fitted with the extrapolated resistance surface derived from F_{ST}, habitat suitability and distance to nearest occupied pond. The conductivity surface was omitted from the nationwide model due to its non-significance within the

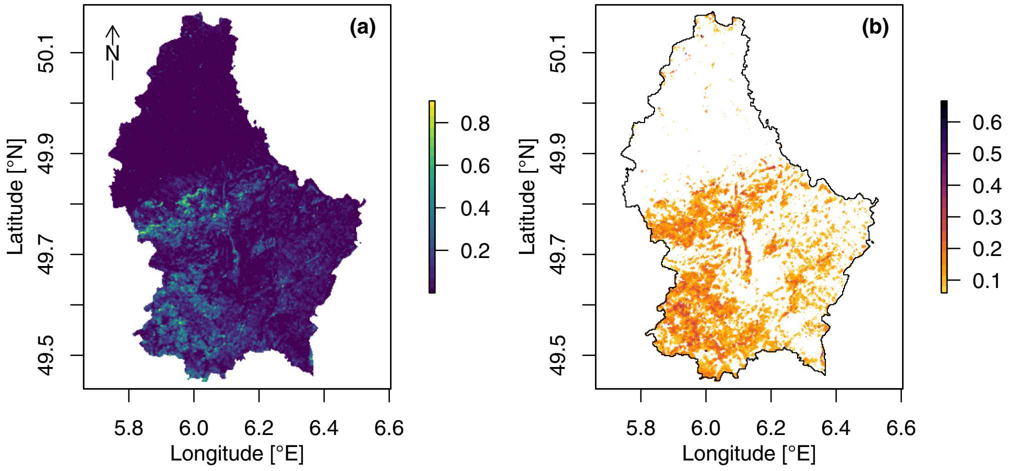

FIGURE 2 Habitat suitability map inferred from final MaxEnt model, with yellow indicating areas of high suitability (a). Areas with high projected gain in habitat suitability when the coverage of standing water bodies is increased through modification of this variable in MaxEnt model input (b). The colour scale shows the difference in habitat suitability ΔHS.

TABLE 3 Summary of generalised additive models (GAMs) fitted with data from the original study area and the whole country.

Variables	HS	DIST	RES	CON	REML	AIC	%Dev
Original study area							
GAM-JostD: HS + DIST + RESJOST + CONJOST	1.57 <0.001	2.36 <0.001	5 0.303	0 0.461	114.32	319.83	36.3
GAM-F_{ST}: HS + DIST + RESFST + CONFST	1.60 <0.001	2.22 <0.001	3 0.006	0 0.699	113.76	313.71	36.7
GAM-pca: HS + DIST + RESPCA + CONPCA	1.57 <0.001	2.37 <0.001	5 0.253	0 0.482	114.26	319.46	36.4
Nationwide							
HS + DIST + RESFST	1.64 <0.001	3.4 <0.001	5 0.565	/	176.23	493.9	34.5
HS + DIST	1.6 <0.001	3.45 <0.001	/	/	180.56	489.03	33.8

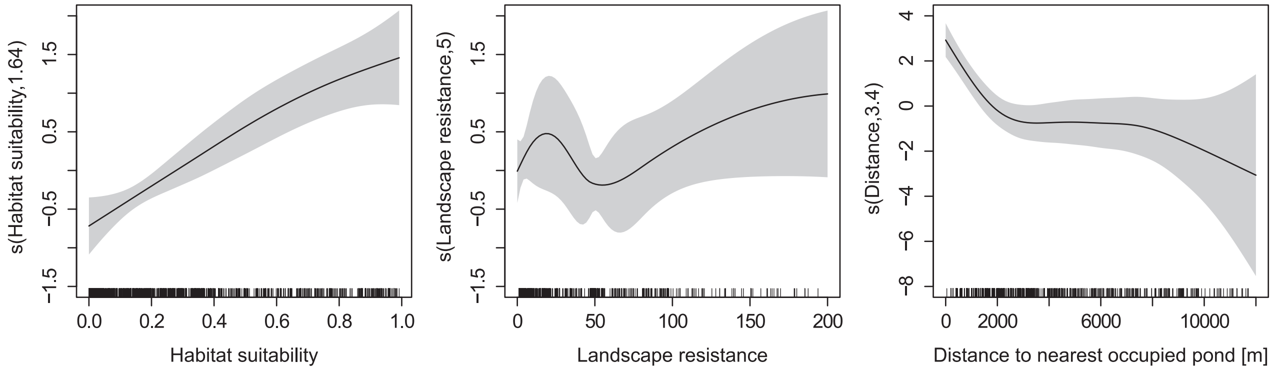

FIGURE 3 Smooth functions fitted in the nationwide GAM. Positive values on the *y* axis indicate a positive effect on crested newt detection probability, with the estimated degrees of freedom provided in the *y* axis label. Tick marks on the *x* axis show the distribution of data points. The shaded area represents the 95% confidence interval.

original study area. The extrapolated resistance surface showed a high correlation with the original optimised surface with Pearson's *r* estimated at 0.84. The nationwide GAM explained 34.5% of observed deviance in the data. As for the reduced model, the distance to the nearest occupied pond was the most important predictor with 73.5%, followed by habitat suitability (22.7%) and landscape resistance (2.3%). When excluding landscape resistance, the percentage of deviance explained dropped slightly to 33.8%. The probability of detecting crested newts decreased strongly within the first 2500 m away from occupied ponds, but increased with habitat suitability (Figure 3). There was no evidence for patterns in model residuals that might have indicated spatial autocorrelation or heteroscedasticity.

To test the potential effect of false absences, 100 GAMs were fitted with 5% or 10% of original absences converted presences. The percentage deviance explained declined from 34.5% to 15.5% (95% CI 15.1–15.9) in the case of 5% of false absences, and to 9.6% (95% CI 9.3–10.0) when assuming 10% of false absences. The importance of the three explanatory variables did not change significantly with the distance to the nearest occupied pond remaining the most influential variable, followed by habitat suitability which lost in significance when assuming the occurrence of false absences (Figure 4). The *p*-value for these variables stayed below 0.001, while landscape resistance did not gain in significance (mean *p*-value = 0.59).

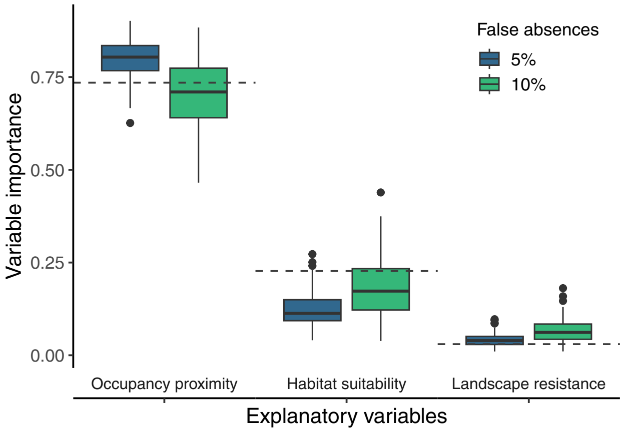

FIGURE 4 The variable importance derived from 100 GAMs, assuming either 5% or 10% of false absences. The dotted lines refer to the estimated variable importance of the original nationwide GAM (assuming no false absences).

3.4 | Spatial prioritisations

Predictions on the response scale derived from the nationwide fitted GAM highlighted the strong correlation with proximity to ponds already occupied by the target species. The highest predicted occurrences of the crested newt were thus located in the southwest and northwest of the Gutland, with only sporadic predictions in the east and an absence in the north of the country (Figure 5a). The map that was produced by solving the spatial prioritisation problem in PRIORITIZR reflected this trend but also considered areas with high ΔHS and excluded areas with high soil imperviousness and in the proximity of motorways (Figure 5b). The addition of a connectivity penalty resulted in solutions with reduced spatial fragmentation, and thus higher contiguity, at the cost of excluding smaller localities (Figure 5c). Given the prominence of motorways in the southwest of Luxembourg, habitats in the northern extent of the species' range were prioritised.

4 | DISCUSSION

The present study aimed to provide an evidence-based framework for habitat restoration and conservation planning for the northern crested newt in Luxembourg. The successful colonisation of human-modified habitats and the persistence of crested newt populations depend on a number of demographic and environmental factors (Cox et al., 2021; Griffiths & Williams, 2000; Halley et al., 1996). It is, therefore, advantageous to consider different aspects of the target's species' biology and ecology to design conservation action plans (McCluskey et al., 2022; O'Brien et al., 2021; Proft et al., 2018). Here, spatial prioritisation recommendations were based on the results of complementary analyses on species distribution, population connectivity and genetic diversity.

4.1 | Conservation genetics

Genetic diversity is a crucial factor in the resilience and persistence of threatened species (Frankham, 2005). Loss of genetic diversity and inbreeding can significantly reduce the adaptive potential and fitness of populations (Frankham, 1998). While the importance of preserving genetic diversity has been widely recognised within various national and international policies, e.g. through the mandate of the Convention of Biological Diversity, genetic aspects are still rarely explicitly incorporated into in situ conservation measures or management policies (Ferreira & Klütsch, 2021; Laikre, 2010). The conservation genetics gap is perpetuated by the mismatch of academic and conservation priorities, limited genetic expertise among policy managers and the restricted communication on how genetic research results can be integrated into policy and management (Ferreira & Klütsch, 2021). In Luxembourg, genetic aspects were found to be less often included in species recovery plans than in other European countries, Australia or the US (Pierson et al., 2016).

Our population genetic analysis was the first nationwide genetic assessment of the northern crested newt in Luxembourg, expanding on the partial population genetic structure reported by Schleimer et al. (2023). Bayesian clustering suggested strong genetic structuring, with closely located breeding ponds forming distinct populations. The increase of genetic differentiation with geographic distance resulted in a pattern of isolation-by-distance, although patterns of differentiation were better explained by a model of isolation-by-resistance (Schleimer et al., 2023). Genetic diversity was found to be high with no signs of inbreeding depression. The fine-scale population structuring and high levels of genetic diversity were in line with previous studies of crested newt population structure in Belgium (Cox et al., 2021), Norway (Haugen et al., 2020) and Scotland (O'Brien et al., 2015).

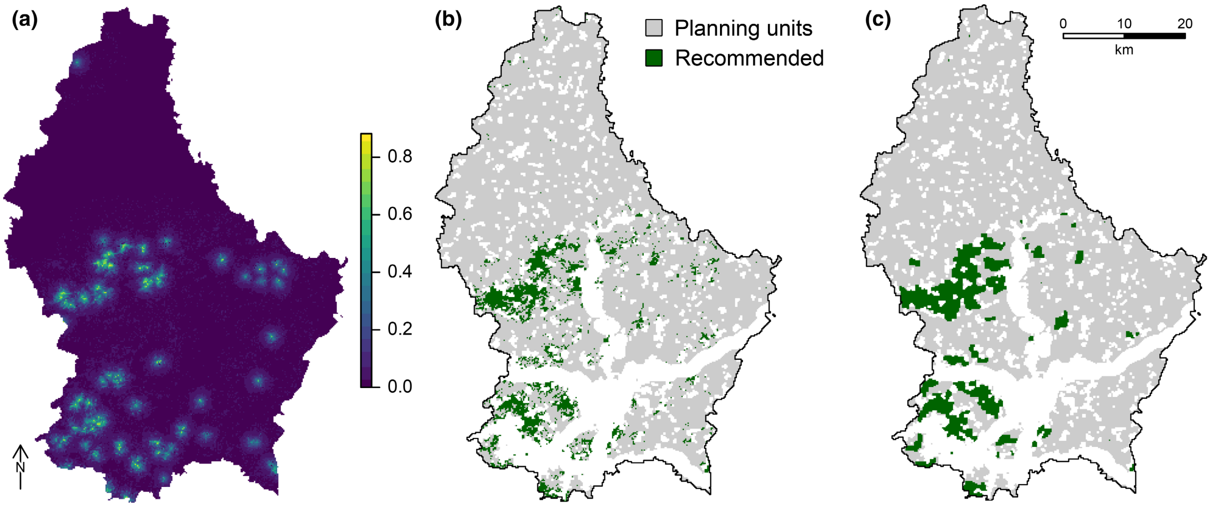

FIGURE 5 (a) Predictions derived from the nationwide GAM (on response scale) where high probability of crested newt occurrence is shown as yellow-green colours. The solution of the spatial prioritisation problem without connectivity penalty (b) and with connectivity penalty (c). Areas of high restoration interest are shown in green. Grid cells that were excluded due to high soil imperviousness (e.g. urban areas) and proximity to motorways were excluded from the grey planning units.

Differentiation across short geographic distances was expected given the limited dispersal capacities of the crested newt (Kupfer & Kneitz, 2000).

The estimation of population-specific F_{ST} highlighted individual ponds (#5, 6, 7, 8, 25, 45) with different genetic composition from the metapopulation in the municipalities of Bissen, Consdorf, Mamer, Schengen and Waldbillig. These sites were also characterised by the lowest levels of allelic richness (A_R=2.43–3.2) and observed heterozygosities (H_O=0.39–0.48) and formed distinct clusters in the Bayesian clustering analysis. A population in Sanem was also identified as a distinct cluster, but with average A_R and H_O. The differentiation and loss of genetic diversity could result from genetic isolation and/or bottleneck events. In view of preserving genetic diversity, these sites should be prioritised in recovery plans for crested newts in Luxembourg, because their isolation could make these subpopulations more vulnerable to extinction. According to Griffiths and Williams (2000), populations of 100–200 individuals have a 27% chance of disappearing over a 50-year period due to demographic and environmental stochasticity. A metapopulation network was found to significantly decrease the extinction risk (Griffiths & Williams, 2000; Halley et al., 1996). Conservation actions should, therefore, focus on maintaining high local abundances and on re-connecting these populations with the metapopulation network. If no suitable breeding ponds are available in the vicinity, the restoration or creation of new breeding habitat is warranted to improve dispersal. With the neutral genetic markers, we could not evaluate whether populations adapted to local environmental conditions in which case the enhancement of population connectivity could erase such local adaptions.

These recommendations are based on the assumption that all surveyed populations are native and that genetic differentiation was not the result of translocated individuals from outside Luxembourg. At the uppermost hierarchical level of clustering (K=2), populations were partitioned into a northern and southern cluster. The introduction of animals from a highly differentiated area would have likely superseded this signal. However, there was at least one known translocation event from the area of Redange to Dudelange (ca. 30 km) that was also evident from the clustering solutions. The Bayesian clustering and the assignment/exclusion test supported the hypothesis that the animals at the 'Trëtterbaach' were introduced from a southern source population, likely originating from the area of Dippach/Sanem. However, given the lack of samples from potential other source populations in neighbouring Germany, as reported by Parent (1984), a more extensive sampling campaign should be conducted with a focus on 'Trëtterbaach' and surroundings. As the first records of this isolated population in the north of the country date back to the 1970s, this population has persisted for already more than 50 years. Unfortunately, only a single sample from this area was available for this study and it was not possible to assess the genetic diversity and level of inbreeding. From a conservation perspective, introduced populations may not warrant the same protection efforts as native ones, although this stance is debatable.

4.2 | Species distribution model

Species distribution models aim to identify the environmental and geological variables that best explain the occurrence of the target species. The importance of ponds and other hydrological variables in our model was expected given the species' reliance on aquatic habitats during its life cycle and is comparable to findings in other studies (Clauzel & Godet, 2020). However, this result was also driven by the biased recording of crested newt presence during the aquatic breeding phase. The majority of species records stem from trapping studies in ponds, with few reports of crested newts during its terrestrial phase. The retained predictor variables are, therefore, not conclusive on the habitat requirements during the terrestrial phase. While the importance of high-quality terrestrial habitats in the vicinity of breeding ponds has been highlighted in multiple studies (e.g. Cox et al., 2021; Denoël et al., 2013), the terrestrial phase of crested newts is understudied compared to the aquatic phase (but see Jarvis, 2016; Jehle, 2000). Because the present study focussed on the restoration of breeding habitats, the bias on the aquatic phase was fitting but a better understanding of the terrestrial ecology could further improve conservation planning.

The SDM highlighted areas of high suitability in accordance with past and current species distribution records. Early reports of crested newts described a relatively common occurrence in the Gutland (south of Luxembourg) and a marked absence in the Ösling (north of Luxembourg), except for the 'Trëtterbaach' population. While the lack of suitable environmental or geological conditions can explain the species' absence from the Ösling, the contemporary patchy distribution in the Gutland, despite large areas with suitable conditions, is most likely the result of anthropogenic pressures. The intensification of agriculture, pesticides, urbanisation and roads reduced available breeding habitat and disrupted connectivity within pondscapes. However, the absence of crested newts from ponds within suitable areas and in close proximity to currently occupied ponds may not only be caused by a lack of connectivity. Oldham et al. (2000) defined a habitat suitability index to predict the presence of the great crested newt, within the UK landscape context, as a geometric mean of 10 suitability indices related to geographic location, pond surface area, pond permanence, water quality, shading, waterfowl presence, fish presence, surrounding pond density, terrestrial habitat and macrophyte cover. This study did not assess pond-specific factors that could have contributed to explaining the absence of the species from certain locations. However, based on the findings from previous studies (Baker & Halliday, 1999; Denoël et al., 2013; Grotz et al., 2018; Hartel et al., 2010), the conservation recommendations for the restoration and creation of crested newt breeding habitat are clear; breeding ponds should be kept free of predatory fish and support macrophyte growth.

4.3 | Spatial prioritisation

The decision on which areas should be prioritised for conservation measures, such as the restoration of ponds, is a trade-off of numerous factors. Conservation practitioners face the demanding responsibility of determining the most effective conservation approach, considering the available scientific knowledge, resources and practical feasibility. In the present study, by far the most important factor explaining pond occupancy was the proximity to other ponds colonised by crested newts. This result is in line with the findings from other studies that highlighted the importance of pond proximity (Clauzel & Godet, 2020; Denoël et al., 2013; Hartel et al., 2010; O'Brien et al., 2021), including previous studies in Luxembourg (Glesener et al., 2022; Grotz et al., 2018). Baker and Halliday (1999) recommended that new ponds should be situated within 400m of known newt ponds to permit natural colonisation. While this result is intuitive given the limited dispersal and high philopatry of the species, it carries important management considerations.

The recommended areas for habitat restoration efforts strongly overlap with the contemporary occurrence of the species. By creating a denser network of ponds in the vicinity of known newt ponds, the resilience of local populations is strengthened and they can act as source populations to colonise new ponds (Denoël et al., 2013). Griffiths and Williams (2000) found that the extinction risk of individual populations was inversely related to the number of suitable breeding ponds within recolonization distance. A metapopulation is less likely to go extinct if individuals can easily disperse among subpopulations. A dense network of well-connected ponds over a small area is, therefore, preferable over sparsely-placed ponds over a wide area. While this approach may not result in a quick range expansion, it ensures a high probability of natural colonisation and higher resilience to extinction. Similarly, proposals to translocate individuals into newly created ponds to promote range expansion risks to leave new populations isolated, vulnerable to inbreeding and with low prospects of persisting in the long term.

The recommendations are conditional on pond occupancy and despite extensive survey efforts over a 10-year period, it cannot be excluded that some presences were missed. While the model permutation suggested that 5%–10% of false negatives would not have affected the overall conclusions of variable importance, missed detections would have affected the final map of recommended areas for conservation efforts. We, therefore, stress the importance of continued monitoring of potential breeding ponds to document the presence and colonisation process of crested newts in Luxembourg.

4.4 | Conservation recommendations

Despite the widely acknowledged importance of evidence-based frameworks, the theory-practice gap persists in species conservation (Cooke et al., 2021). One contributing factor to the research-implementation gap is the lack of clear communication on how scientific results can be used to inform policy and management (Ferreira & Klütsch, 2021). This study combined results from SDMs, landscape genetics and population genetics into a common framework to make habitat restoration recommendations for the crested newt in Luxembourg (Box 1). The National Plan for Nature Protection (MECDD, 2023), adopted in January 2023 by the Government in Luxembourg, sets out to restore 174 water bodies and create 350 new standing water bodies by 2026, and doubling these numbers by 2030. By using the priority areas identified in the present study as guidance for spatial prioritisation, conservation practitioners can target their efforts to improve the conservation status of the protected crested newt, in accordance with the recommendations outlined in the Species Action Plan (Proess & Schneider, 2018).

BOX 1

- Conservation priority should be given to subpopulations of ponds in the municipalities that were genetically differentiated and/or had lower genetic diversity.
- The map in Figure 5 should inform conservation planning for the crested newt in Luxembourg in order to meet the goals set by the National Plan for the Protection of Nature (PNPN3).
- A dense network of well-connected breeding ponds should be favoured over a vast network of sparsely spaced ponds.
- Frequent monitoring should be conducted to detect new presence and colonisation events.
- Translocations that would result in highly isolated populations are not recommended.

AUTHOR CONTRIBUTIONS

Alain C. Frantz and Lisette Cantú-Salazar conceived the idea and design of the study; Amanda Luttringer conducted the field and laboratory work; genetic data were analysed by Anna Schleimer, Amanda Luttringer and Alain C. Frantz; Lisette Cantú-Salazar fitted the species distribution models; Anna Schleimer led the writing of the manuscript with input from all co-authors. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/2688-8319.12310.

DATA AVAILABILITY STATEMENT

Geo-referenced multilocus genotypes of all 1068 samples were deposited on figshare: https://doi.org/10.6084/m9.figshare.25144220.v1 (Schleimer et al., 2024). Species occurrence records are available from the RECORDER platform of the National Museum of Natural History, Luxembourg (MNHNL, 2024), and can be accessed through the mdata.mnhn.lu platform. An online map with the priority areas of conservation from Figure 5 is archived in Zenodo: https://doi.org/10.5281/zenodo.10630107 (Schleimer, 2024), available in GitHub: https://github.com/Bphysalus-anna/Tcristatus.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Candidate environmental predictor variables for MAXENT modelling.

Figure S1. Estimates of pairwise F_{ST} (Weir & Cockerham, 1984) between ponds with $N \geq 14$ samples.

Figure S2. Log likelihood for different cluster solutions for $K=1$-20 as inferred from STRUCTURE.

Figure S3. Individual admixture levels estimated in STRUCTURE for $K=2$ (A), $K=6$ (B) and $K=18$ (C) for the northern crested newt in Luxembourg.

Figure S4. Clustering solutions from STRUCTURE at $K=18$ (A) and BAPS at $K=20$ (B).

Figure S5. Tests of collinearity among predictor variables.

Figure S6. Smooth functions fitted in the study-area GAM.

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