Interventions combining species, habitat and genetic information reverse a downward trend in threatened peripheral amphibian (*Triturus cristatus*) populations

David O'Brien[1, 2], J.E. Hall[2], A. Miró[3], K. O'Brien[3], M. Falaschi[4] & R. Jehle[1]

1. University of Salford, School of Science, Engineering and Environment, Salford M5 4WT, UK.

2. Scottish Natural Heritage, Great Glen House, Leachkin Road, Inverness IV3 8NW, UK.

3. Highland Amphibian and Reptile Project, c/o Woodlands, Brae of Kinkell, Dingwall, IV7 8HZ, UK

4. University of Milan, Department of Environmental Science and Policy, Via Celoria 26, 20133 Milan, Italy.

Corresponding author: david.obrien@nature.scot

Author contributions

DOB, JH, AM and RJ conceived the ideas and designed methodology; field work including data collection was carried out by DOB, JH, AM and KOB; DOB led on project management; AM, MF and DOB analysed the data; DOB and JH led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication

Abstract

Conserving genetic diversity in wild species is vital for preserving adaptations to local environmental conditions. We conducted a habitat creation project for a flagship European Protected Species of amphibian (northern great crested newt *Triturus cristatus*) at its north-western range edge in the Scottish Highlands, combining existing knowledge about the species' local habitat requirements and the genetic structure of existing populations. Reconciled by a spatial connectivity model, we determined the best locations for the creation or restoration of 25 ponds to facilitate their natural colonisation, and to reconnect areas with similar genetic makeup whilst preserving differences between populations belonging to separate genetic clusters. A dynamic occupancy model based on data derived from standardised surveys revealed that both detection and occupancy probabilities strongly depended on habitat features known to be locally favourable for *T. cristatus.* At least 12 of the 25 new ponds were colonised within six years, representing a 26% increase in number of breeding ponds in the region and offsetting a gradual decline in number of pre-existing breeding sites recorded over the last 3 decades. Combining genetic evidence, spatial modelling and historical knowledge with local stakeholder engagement led to a habitat creation and management scheme that has met its immediate and evidence-based conservation goals in a way which is transferable to a range of different systems.

Keywords

genetic diversity; pond creation; range edge; connectivity map; evidence-based conservation; dynamic occupancy model

1. Introduction

Recent global (Díaz et al., 2019a; b) and continental-level (European Environment Agency, 2019b) reports have re-iterated the unprecedented current pressures on biodiversity. Whilst no groups are immune to such pressures, amphibians are the most severely impacted vertebrate taxon and, after cycads, the second most threatened major taxon assessed by the IUCN (Díaz et al., 2019a; Hoffmann et al., 2010). Changes in land use, particularly habitat loss and degradation, have long been proposed as major factors in amphibian decline (e.g. Cooke and Ferguson, 1976; Cushman, 2006). Their limited powers of dispersal and, in many cases, reliance on focal breeding sites, makes them particularly vulnerable to isolation, leading to genetic problems such as inbreeding and loss of diversity through drift (Allentoft and O'Brien, 2010).

The Convention on Biological Diversity recognises three strands to biodiversity: species, ecosystems and genetic variation (CBD, 1992). However, although genetic variation is crucial to ensuring that species can adapt to environmental changes, it is rarely explicitly incorporated as an evidence base into practical *in situ* conservation measures (Hoban et al., 2020; Pérez-Espona and ConGRESS Consortium, 2017). A formal way of bringing together these three strands of biodiversity is the establishment of Gene Conservation

Units (GCUs), which are areas managed to allow adaptation to environmental change through natural selection on the standing levels of local genetic variation (de Vries et al., 2015; Minter et al., 2021).

Within Europe, the northern great crested newt *Triturus cristatus*, a pond-breeding amphibian, has been designated a conservation priority, with heavy investment in conservation and research (Jehle et al., 2011). Population persistence of *T. cristatus* is associated with connectivity of demes (Halley et al., 1996; Griffiths et al., 2010; Griffiths and Williams, 2000), and the genetic structure of local populations is determined by the terrestrial habitats surrounding ponds (Cox et al., 2021).

Separated from the main British distribution by about 70 km, the Scottish Highlands has the most northerly *T. cristatus* populations within the British Isles, whose native status has only been clearly established in the last six years (O'Brien and Hall, 2012; O’Brien et al., 2015). Local habitat preferences differ considerably from the species’ core distribution, being similar to those in Norway (Skei et al., 2006), even though a range of habitat types favoured elsewhere, such as broadleaved woodland, are present within its Highland range (Miró et al., 2017). Using information derived from polymorphic microsatellite markers, O'Brien et al. (2015) described four genetic clusters within the Highlands, separated from each other by intervening unfavourable habitats such as fast-flowing rivers or acidic heathland. While ponds within clusters were characterised by low levels of genetic differentiation between them, they were also separated from each other by geographic distances exceeding known *T. cristatus* movement abilities (about 1.5 km, e.g. Haubreck & Altrichter, 2016). This observation is consistent with pond loss due to changes in agricultural practice during the 20th century (see also O'Brien and Hall, 2012), and suggests the potential for strengthening local metapopulations by the creation of additional breeding habitats.

In this paper we report on an evidence-based conservation intervention project to enhance the available breeding habitat for *T. cristatus* fringe populations in the Scottish Highlands. The main aims are to (i) reconnect *T. cristatus* populations of similar genetic structure (those belonging to the same cluster but separated by 20th century loss of ponds) by creating new ponds in areas with favourable habitat, (ii) preserve genetic distinctiveness of local genetic clusters as reflected in natural barriers to gene flow, and (iii) enhance populations which were considered to be most at risk of future population extinction due to environmental deterioration, stochastic processes due to small population sizes or genetic erosion. In order to achieve this, we evaluated the location of new habitats using a landscape connectivity map, and quantified the pond-specific occurrence of *T. cristatus* using a dynamic occupancy model based on targeted surveys. The paper builds upon existing descriptions of colonisation of the newly created habitats by local amphibian communities in conjunction with local stakeholder involvement (O'Brien et al., 2021), and should form a useful case example for the conservation of isolated metapopulations of threatened taxa.

2. Methods

2.1 Study area

The study area is situated within the Scottish Highlands (UK) adjacent to the Moray Firth and Aviemore (Figure 1). This area has a relatively mild climate for its latitude (McClatchey et al., 2014), and encompasses all native *T. cristatus* populations in the Scottish Highlands (O'Brien et al., 2015). The present study considers 120 ponds, comprising all 46 ponds where *T. cristatus* is known to have occurred since 1990, 49 control ponds without known *T. cristatus* occurrence, and 25 intervention ponds (consisting of 20 new and 5 restored ponds). Control ponds were selected within the same 10 x 10 km^2 grid as the known *T. cristatus* ponds (4˚35'-3˚35'W, 57˚38'-57˚11'N) using a random number generator (Miró et al., 2017; O'Brien et al., 2017). Ponds ranged from 4 - 168,000 m2 and included those with and without fish. For the present work, we use the term 'metapopulations' to refer to groups of ponds which allow colonisation by immigrants from other ponds within the same metapopulation (Brown and Kodric-Brown, 1977; Hanski, 1998).

2.2 Survey and habitat intervention

As a foundation for this study, we built a database of yearly *T. cristatus* detection/non-detection/no survey (1/0/NA) events over 11 sampling years from 2010 to 2020 (Pollock, 1982). Surveys were carried out following the protocol used for the National Amphibian and Reptile Recording Scheme (NARRS, Wilkinson and Arnell, 2013), a UK-wide citizen science survey programme for widespread species. In brief, surveys are carried out during the breeding season (April to June in Highland Scotland) using four methods (daytime egg searching and netting, night-time torching (flash-lighting) and trapping). Up to three visits are made per year, but survey ceases once presence is established or if the pond has fully dried up. The Covid-19 pandemic meant that survey effort in 2020 was limited to ponds within permitted distances from the authors' homes.

Conservation interventions took two forms: remedial management of three ponds with existing populations, and the creation or re-creation of 25 ponds across three of the known population clusters (Contin/Strathpeffer, Black Isle and Forres, see Figure 1). The remedial management was carried out between 2012 and 2014 by a combination of volunteers and government agency staff, and involved removing colonising willow *Salix spp*, deepening sections to reduce desiccation risk, or temporary draining to extirpate non-native European perch *Perca fluviatilis*. Pond (re)creation work was carried out in Autumn-Winter 2014-15 for 24 ponds, and during Winter 2017-18 for one additional pond (Table A1 in the Appendix).

A first pond creation scheme was carried out at two wooded sites within the Contin/ Strathpeffer pond cluster (Figure 1), managed by Forestry and Land Scotland (the government agency responsible for managing Scotland's national forest estate). We surveyed the woodland for potential pond sites, focusing on damp areas without established trees, and with flora suggestive of less acidic conditions (e.g. with *Eriophorum latifolium* and *Juncus spp*, but lacking *Narthecium ossifragum* and *Trichophorum germanicum*). We created eleven ponds and recreated two previously existing ponds. All ponds were adjacent to mixed *Pinus sylvestris* – *Betula* woodland (EUNIS category G4.4, European Environment Agency, 2019a), and on humus-rich iron podsols or brown earths.

A second scheme was carried out in partnership with local farmers and a golf club (O'Brien et al., 2021). This led to the creation of one new pond at Forres golf course, and a network of 11 ponds (including re-creation of three old ponds) in farmland on the Black Isle in winter 2014-2015 (Figure 1). Eight of these ponds were adjacent to mixed *Pinus sylvestris* – *Betula* woodland, and the remainder, including the golf course, were close to other suitable habitat according to Miró et al. (2017). The golf course and seven of the farmland ponds were on humus-rich iron podsols, the others on non-calcareous gleys.

Ponds were generally designed and constructed according to *T. cristatus* local habitat preferences, such as slightly sloping banks, high terrestrial habitat diversity and presence of adjacent mixed woodland (following Miró et al., 2017, see also Table 1). All excavation work was carried out using 13t excavator on low pressure treads as recommended in McKinnell et al. (2015). Ponds were profiled to give shallow margins, likely to warm quickly in spring and offer easy egress for metamorphs, with ≤1.5m deep sections to reduce desiccation risk and to provide ice-free over-wintering sites for *T. cristatus* and other amphibians which may hibernate in ponds (*Rana temporaria, Lissotriton helveticus*).

2.3 Connectivity mapping

To assess the location of our intervention ponds in view of their function as stepping stones, we first created a suitability map with the software Maxent, using occupied ponds as presence localities (following Phillips et al., 2006). Three ponds near Aviemore were excluded, as they belonged to a cluster which was not part of the intervention scheme, and which is separated from the remaining ponds by unfavourable habitat and a distance by far exceeding the species' known dispersal range. Landscape variables were retrieved from the EUNIS land cover map of Scotland (SEWeb, 2014), with the initial 56 habitat categories reclassified into ten biologically relevant variables as described in Table A4. Among these variables, we considered 7 categories for the analyses: 1) constructed areas with high density, 3) constructed areas with low density 3) cultivated areas, 4) woodlands, 5) grasslands, 6) heathlands, 7) mires, bogs and fens. These broad categories do not include for example pond features, and thus may over-estimate suitability. Because the importance of terrestrial habitat surrounding *T. cristatus* breeding ponds decreases with distance (Cox et al., 2021) and based on commonly documented migration distances (Baker and Halliday, 1999; Kupfer and Kneitz, 2000; Jehle et al., 2011), we calculated the percentage cover of each variable at a radius of 400 m around each pond. In a second step, the seven variables were aggregated from the initial resolution of 10 × 10 m to a resolution of 100 × 100 m. To assess relationships between species occurrence and environmental variables with Maxent, we considered linear and quadratic terms, using 10,000 pseudo-absences or background points which were randomly selected from the study area.

In order to assess the fit of the suitability model we performed cross-fold validation, splitting the dataset into five parts and using one at a time for model testing and four parts for model training (Merow et al., 2013); the average cross-validated value of area under the receiver operating curve was 0.871. We then averaged the five Maxent models and projected them on the environmental layers to obtain a suitability map. To obtain a conductivity map, we further rescaled the suitability map between zero and 1,000 and set lakes and marine coastal habitats as not permeable. This map was used to run a connectivity model with Circuitscape 4.0 (https://circuitscape.org/). Circuitscape was run in pairwise mode, and the cells where the 43 occupied ponds are located were used as sources to identify possible habitat corridors (McRae et al., 2008).

2.4 Dynamic occupancy model

To understand occupancy and colonisation, we used dynamic (or multi-season) occupancy models (MacKenzie et al., 2003), which allow one to relate environmental covariates to metapopulation parameters and account for imperfect detection (e.g. Fardell et al., 2018; Haggerty et al., 2019; Vanek et al., 2019; Villena et al., 2016; Guillera-Arroita, 2017). We considered first season occupancy probability (ψ), pond colonization probability (γ), probability of extinction from pond (ε), and species detection probability (ρ). The model is able to deal with gaps from incomplete surveys, such as those resulting from restrictions during the Covid-19 outbreak in 2020. Following Burnham and Anderson (2004), we incorporated twelve habitat variables of biological relevance (Miró et al., 2017) to fit model parameter estimates and to assess the effect of conservation interventions on *T. cristatus* metapopulation parameters across the study period (see Table 1).

To implement the model, we generated the year-specific binary factor “intervention pond yes/no” (Table A1 in the Appendix). Each constructed pond was coded as “NA” before the construction year, and as “yes” from its construction onwards. Each restored pond was coded as “no” before the restoration year and as “yes” from its restoration onwards. The ponds not involved in restoration works were coded as “no” across the entire study period. We then added eight site-specific habitat characteristics that had been shown to be the most important for *T. cristatus* occurrence in the Scottish Highlands in a previous study (Miró et al., 2017): adjacent mixed woodland, organic mud pond substrate, frequency of desiccation (defined by surveyor after discussion with land manager), terrestrial habitat richness, fish presence (a negative factor), aquatic vegetation pond coverage, slightly sloping bank pond proportion, and macroinvertebrate richness (see also Table 1). Habitat characteristics were surveyed during the 2014 breeding season for the occupied and control ponds (Miró et al., 2017), while intervention ponds were surveyed during 2016 and 2019 breeding seasons specifically for this study. For factors which change over time, we used the value of the survey results for the year in which *T. cristatus* was first recorded. Additionally, to investigate the role of connectivity, we generated the site-specific covariate "nearest pond occupied in 2010" by computing the linear distance of each pond to the nearest pond occupied by *T. cristatus* in the initial breeding season of the study period (2010). Linear distance was obtained from a GIS using 1:25 000 maps from the British mapping agency Ordnance Survey. Finally, to assess the existence of temporal patterns across the study period, we generated one yearly categorical and one numeric variable determined by the year of origin of the response data within the study period from 2010 to 2020 (Table 1). Whilst all intervening ponds were surveyed for *T. cristatus*, the possibility of negatives cannot be discounted and the species might occur in some ponds where it was classed as absent (see also Griffiths et al., 2015).

We identified the best dynamic occupancy model by fitting a set of candidate models, adding covariates to the single parameter regressions shown in Table 1 (backward selection) and retaining the covariates which decreased the model Akaike Information Criterion estimator AICc corrected for small sample sizes (Akaike, 1974; Burnham and Anderson, 2004). Model parameters were fitted in the order detection probability (ρ), initial occupancy probability (ψ), colonization probability (γ), and extinction probability (ε). Candidate models were ranked in ascending AICc order (Table A2). Then, we computed model back-transformed coefficient estimates for the covariates from the top candidate model with ∆AICc<2 (Burnham and Anderson, 2004). We illustrated the strength and direction of covariate effects by drawing the predicted relationship of each selected covariate along its range with the modelled parameters (Fiske and Chandler, 2011).

Dynamic occupancy models were fitted in *R* (*R* Core Team, 2018) using the function *colext* and complementary functions in the package *unmarked* (Fiske and Chandler, 2011). Numerical covariates were standardized to zero mean and unit variance to correct their heterogeneous dimensions and to avoid algorithm optimization problems (Gelman and Hill, 2007). We also examined the covariates dataset for linear dependencies by computing Variance Inflation Factors (VIF), which were <1.5 in all cases (well below 3 to 5, the threshold indicative of worrisome collinearity for regressions, Zuur et al., 2009). Finally, we assessed goodness-of-fit of the top model by computing the sum of squared estimate errors (SSE), Chi-square, and Freeman-Tukey statistics based on 5000 parametric bootstraps (following MacKenzie and Bailey, 2004; Fiske and Chandler, 2011).

2.5 Occupancy trend

We computed the derived yearly occupancy probability from 2010 to 2020 from the modelled estimates of initial occupancy probability (ψ), pond colonization probability (γ) and pond extinction probability (ε) following MacKenzie et al. (2003). Since we had surveyed a proportion of the occupied ponds each year across the study period, we used derived yearly occupancy estimates from the proportion of the sampled ponds that are occupied (smoother estimates; Weir et al., 2009) and computed standard errors based on 1,000 non-parametric bootstraps. We tested the effect of conservation interventions on the number of populations by computing analyses of covariance (ANCOVA) on the yearly derived occupancy as response variable against Year (from 2010 to 2020) as numeric independent variable, and period of time before (2010-2014)/after (2015-2020) interventions as factor. We included the interaction term Year:Period before/after to test if the trend in numbers of populations (i.e. Year slope) showed differences between both periods.

3. Results

3.1. Survey and habitat intervention

In the three ponds subject to remedial management, adult populations in one case returned to around former levels (increasing from ‘Low’ to ‘Good’ using the criteria of JNCC 1989). In the other two ponds, numbers of adults seemingly remained stable; it is likely that larval survivorship improved, but both are part of well-connected metapopulations and we have not attempted to disentangle their population dynamics.

Triturus cristatus had colonised 12 of the 25 newly created or recreated ponds by 2020 (i.e. within six years of creation), with one pond (B8) colonised in the first breeding season after construction. In total, we observed 368 *T. cristatus* detection events, 2810 non-detection events, and 782 NAs, based on up to three site surveys in a given year. *T. cristatus* largely colonised new sites close to existing breeding ponds most rapidly. The observed colonisation distances were 219 – 592m from pre-existing ponds, with one exceptional pond 1,840 m from the nearest known putative source population. By 2020, intervention ponds made up one quarter of *T. cristatus* breeding sites in the Highlands. One of the intervention ponds now holds one of the largest breeding populations of *T. cristatus* in our study area (G02, Appendix Table A1), with no detectable drop in population numbers at the putative source pond. Engagement with land managers has also led to an increase in the number of ponds in sites with conservation plans agreed with the owner (from eight in 2014 to 21 in 2020, O’Brien et al., 2021), and those where the owner has agreed to manage the land sympathetically in a less formal manner (from four to five).

3.2 Connectivity mapping

The connectivity map derived through the Maxent model (Figure 2) is broadly in line with the genetic clusters found in O'Brien et al. (2015). Connectivity within clusters is high, decreasing with increasing distance from them. One exception is that the map suggests high habitat connectivity between the genetically rather isolated Black Isle and West Inverness populations. It also suggests low connectivity within the putative East Inverness populations, as well as low between Forres and the adjacent populations.

3.3 Dynamic occupancy model

The model selection procedure showed support for a single top model (ΔAICc <2; Table A2 in the Appendix). It suggested that the *T. cristatus* detection probability (ρ) ranged from 0.28 to 0.48, increasing with aquatic vegetation coverage and decreasing with fish presence compared to fish absence (means of 0.28 and 0.38 respectively; Table 2; Figure 3). Initial occupancy probability (ψ) increased with adjacent mixed wood coverage (0.20 to 0.86), organic mud pond substrate (0.17 to 0.64) and terrestrial habitat richness (0.04 to 0.94), and decreased with the frequency of desiccation (0.02 to 0.45), the distance to the nearest occupied pond (0.05 to 0.42), and the absence rather than presence of fish (mean 0.38 and 0.22 respectively; Table 2; Figure A2 in the Appendix). Pond colonization probability (γ) decreased with distance to the nearest occupied pond (0.00 to 0.99, being >0.5 for distances <470m), and increased with conservation intervention (mean 0.23 and 0.00 respectively; Table 2; Figure 3). No covariates were retained for pond extinction probability (ε). The bootstrap p-values based on the SSE, Chi-square and Freeman-Tukey statistics were 0.89, 0.82 and 0.64 for the best model (fm15), respectively, suggesting that it provided an adequate fit to the data (Figure A3 in the Appendix). Due to the small number of recreated ponds (n=5), it was not possible to make a meaningful comparison between them and those constructed from new.

3.4 Occupancy trend

The estimated number of occupied ponds yielded by the dynamic occupancy model was 42 in 2010, decreasing slightly to 40 in 2015 before increasing to 49 in 2020 (Figure 4 and Table A3 in the Appendix). The period before interventions (2010-2014) showed a negative trend (Year slope of -0.41 ±SE 0.08, *P*= 0.013; Figure 4), while the period after interventions (2015-2020) showed a positive trend (Year slope of 1.46 ±SE 0.23, *P*= 0.003; Figure 4 and Table 3). The ANCOVA (3 on 7 DF, P< 0.001) supported differences between the periods before and after interventions, with a significant slope for Year:period interaction of -1.88 ±SE 0.30 (*P*= 0.001, Figure 4).

4. Discussion

This study aimed to evaluate the effectiveness of a habitat restoration programme for the conservation of *T. cristatus* in the Scottish Highlands, which sought to maintain distinct local genetic lineages with the avoidance of deleterious genetic processes linked to isolation. The intention is that this would foster adaptation to environmental change through natural selection on the standing levels of local genetic variation, as intended for GCUs as proposed by Minter et al. (2021). In order to do this, we considered barriers to movement, as well as pond-specific biotic and abiotic factors that may be linked to colonisation. Our intervention sites prevented a steady decline in the number of ponds occupied and resulted in an increase in the number of ponds managed wholly or partly for conservation of *T. cristatus*. As might be expected with a declining species, the original breeding sites were often isolated from each other and separated by unfavourable habitat prior to the intervention. Using a detailed occupancy model which takes detectability into account, our results show that the programme has been effective in increasing the number of breeding ponds used by *T. cristatus*.

4.1 Effectiveness of the habitat interventions for the conservation of *T. cristatus*

Our knowledge of local habitat preferences led us to situate most of the intervention ponds in areas of high terrestrial habitat richness, near to mixed *Pinus sylvestris* – *Betula* woodland, a habitat strongly associated with persistence of *T. cristatus* in the region (Miró et al., 2017). This relationship between habitat and initial occupancy was also confirmed by the dynamic occupancy model of the present study. In the case of the original Forres Golf Club pond, which held an isolated and declining population with low allelic richness (O'Brien et al., 2015), the priority was to construct a suitable pond which might also form a stepping stone to nearby existing woodland ponds. Our findings confirmed previous studies on *T. cristatus* that have highlighted the negative impact of fish (Figure 2.b1; reviewed in Jehle et al., 2011). Whilst *T. cristatus* in the Highlands also demonstrated a strong association with well-vegetated ponds (Miró et al., 2017; Figure 3.b2), we preferred to allow plants to colonise the intervention ponds naturally rather than planting them. Colonisation by helophytes was rapid, including species typically used for egg-laying by *T. cristatus* (such as *Epilobium* spp, *Myosotis* spp and *Glyceria fluitans,* see Miró et al., 2017).

The rapid colonisation of ponds by *T. cristatus* suggests that our work has produced attractive and accessible breeding habitat. The broadly linear colonisation process from occupied sites was expected, because ponds were constructed in a way that would allow sequential colonisation, and ultimately enable gene flow within reconnected metapopulations. All but one of the observed *T. cristatus* colonisation distances (Figure 3.a2) were at a similar range compared to other studies (Baker and Halliday, 1999; Kupfer and Kneitz, 2000): 219 – 592m from pre-existing ponds. The single outlier pond (C17) at a distance of 1,840m may have been colonised from an extensive area of local wetland containing individuals that remained undetected during surveys. Due to resource constraints, a pond intended as a stepping stone was only constructed in 2017 (B07, Appendix Table A1). It seems likely that this has slowed down colonisation in the area, although breeding by *T. cristatus* was recorded there and in neighbouring new ponds in 2020.

Conservation of *T. cristatus* is best achieved by improving the functioning of their metapopulations (Jehle et al., 2011). The sets of new ponds in the three pre-existing pond clusters (Contin/Strathpeffer, Black Isle and Forres; Figure 1) were created to increase the available breeding habitat in specific locations to reinforce local metapopulations. Griffiths and Williams (2000) found that the likelihood of individual populations going extinct is inversely related to the number of suitable breeding ponds within recolonization distance, and Halley et al. (1996) found that even large populations located over 3km from the nearest source pond had a greater than 95% chance of extinction within 20 generations. Even where resources meant we were only able to construct a single new pond at one cluster (Forres), these studies suggest that extinction risk might nevertheless be reduced in this area from over 75% to well below 50% over 50 years, thus potentially stabilising the eastern edge of the species' local range.

4.2 Connectivity

Perhaps counter-intuitively, the need for closely adjacent ponds is greatest in areas where the habitat is only moderately favourable, and thus pond-level extinctions can be expected. The modelled low connectivity between Forres and the other populations suggests that additional pond creation may be needed in this area. O'Brien et al. (2015) found low genetic diversity in this population, consistent with long-term isolation. Further breeding sites have subsequently been discovered in the Forres area (Miró et al., 2017), however at a distance exceeding the recorded maximum recorded migration distance of *T. cristatus* (1 650 m, Haubrock & Altrichter, 2016). The newly created pond reduces the distance between them and the original golf course pond, thus supporting the likelihood of gene flow. Whilst these populations have persisted up to now, an increased likelihood of drought as a result of climate change may lead to more frequent pond-level extinctions (Kirkpatrick Baird et al., 2021), and thus the need for greater attention to meta-population dynamic processes in this cluster.

The connectivity map shows high habitat connectivity between the Black Isle and West Inverness populations. However, actual connectivity for *T. cristatus* is likely to be lower, as the model fails to take into account an intervening fast-flowing river (the Beauly) with high salinity levels close to its mouth, combined with a lack of local ponds that could serve as stepping stones.

4.3 In situ genetic conservation

Knowledge of the genetic structure of the local *T. cristatus* populations served as a basis for the planning of our interventions. Although the influence of pond (re)creation on the local standing amount of genetic variation remains to be measured, we assume that our interventions have been beneficial to the overall levels of genetic diversity: O’Brien et al. (2015) highlighted low allelic richness in the Forres population, suggesting risks relating to genetic erosion. Our work has led to an increase in the number of breeding ponds while simultaneously reducing the inter-pond distance, promoting migration and thus possibly gene flow. Ponds in the Contin/Strathpeffer cluster are now better connected, as are those in the Black Isle cluster. O’Brien et al. (2015) had found low F_{ST} values between ponds in the Black Isle cluster despite an absence of ponds between them, which is consistent with the known loss of agricultural ponds in the area during the 20th century. The increased number of breeding ponds in both areas should decrease the risk of loss of genetic diversity from metapopulation demographic processes while maintaining the locally distinctive population. Given the low connectivity between the East Inverness set of populations, further studies to establish the local level of genetic diversity would be useful to guide possible future intervention. Taken together, the conservation measures for *T. cristatus* in the Scottish Highlands provide a useful case study of an approach that would meet the criteria for GCUs as proposed by Minter et al. (2021).

4.4 Breeding pond occupancy

The dynamic occupancy model (Figure 4) demonstrates that the historic decline of *T. cristatus* described in Miró et al. (2017) would have continued in the absence of intervention. Between 1990 and 2012, evidence for local extinctions was observed for nine ponds and severe declines were recorded in a further four ponds, principally as a result of succession, desiccation or fish introduction (Miró et al., 2017), and a further pond was lost in 2019. As a result of our interventions, the derived overall occupancy trend is positive. The 12 newly established *T. cristatus* populations have increased the total number of sites in the Highland region to 56 (records since 1990; NBN, 2019). The dynamic occupancy model identified distance between ponds, desiccation and fish presence as negatively associated with initial occupancy, confirming the value of the intervention work carried out. However, it should be noted that occasional pond desiccation, around one year in ten, appears to be favourable to *T. cristatus* (provided re-colonisation from nearby ponds is possible), as it stops the establishment of fish and reduces numbers of some invertebrate predators (Griffiths 1997; O'Brien et al., 2017; Oldham et al., 2000).

Our findings mirror Europe-wide causes of decline in *T. cristatus* reviewed in Jehle et al., 2011 although, unlike that review, no evidence has been reported of declining water quality in the Highlands (SEWeb, 2020), and water quality in all ponds with *T. cristatus* in our study was high (Miró et al., 2017). Furthermore, two of the ponds suffering loss of *T. cristatus* were newly created fishponds which only held *T. cristatus* briefly before fish became established, and another pond was found to have been recolonised in 2018. Therefore, the overall outlook based on pond occupancy seems favourable.

4.5 Implications for conservation management of *in-situ* populations

We believe that the success of this project results from the combination of habitat, genetic and human societal factors. Before any landscape-scale intervention for the benefit of range edge or long-isolated populations, we recommend investigation of local habitat preferences, rather than relying on information from a species' core range (see also O'Brien et al., 2021). Further, we recommend quantification of local genetic patterns to counterbalance the risks of local genetic erosion against the potential loss of locally adapted variation through reconnecting long-isolated populations. Rather than considering individual populations as main units, habitat connectivity analysis, in our case combined with historic data, can be used to target intervention sites that augment local metapopulations without disrupting natural ecological and genetic partitions.

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Ethical statement

Survey work was carried out under licence from Scottish Natural Heritage and adhered to their ethical and biosecurity guidance.

Competing interests

The authors declare that they have no competing interests.

Data availability

Original species records, including records of non-target species are publicly available via the National Biodiversity Network (https://nbnatlas.org/).

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