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The ecology and evolution of the northern pool frog (*Pelophylax lessonae*) and the importance of its conservation in England

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Masters of Research in Biosciences

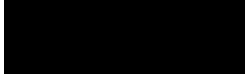
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Summary:

Pool frogs (*Pelophylax lessonae*) in northern Europe provide an intriguing opportunity to study a species complex in a conservation context. They form part of a species complex with the marsh frog (*P. ridibundus*) and the edible frog (*P. kl. esculenta*), with hybrid populations common across mainland Europe. However, small, isolated, single-species populations of a distinct northern clade exist along the coasts of the North and Baltic seas, including an extinct, and since reintroduced, population in England. These northern populations are of high conservation priority due to their declining numbers and ecological distinctness. This study employed maximum likelihood phylogenetic methods on 50 cytochrome B sequences to infer a phylogeny for the species, supporting previous theories that suggest pool frogs originated in the Italian Peninsula before migrating into Central Europe, where they rapidly spread and diversified. Sequences from Denmark and Italy appear ancestral, though limited genetic structure complicates further colonization inferences. Species Distribution Models (SDMs) assessed the climatic niche of pool frogs across Europe, revealing that northern populations occupy a narrower climatic niche, confined to low-lying coastal areas with low precipitation, compared to their hybrid counterparts. SDMs predict suitable climates for northern pool frogs in eastern and southeastern England, aligning with historical records. Conservation efforts should focus on restoring the species to East Anglia, southern Lincolnshire, and the Thames estuary, while mitigating the risks of hybridization with non-native *Pelophylax* species.

Declarations and Statements

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.


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This thesis is the result of my own investigations, except where otherwise stated.


Other sources are acknowledged by footnotes giving explicit references. A

bibliography is appended.

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The University's ethical procedures have been followed and, where appropriate, that

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Health and Safety / Risk Assessments

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Table of Contents

1. Introduction	7
2. Methods.....	11
2.1 Phylogenetic Tree Reconstruction	12
2.2 Climate niche modelling	13
2.3 Niche similarity calculations.....	19
3. Results.....	20
3.1 Phylogeny of Pool Frogs.....	20
3.2 Climate Niche Models.....	22
3.3 Niche Similarity.....	25
3.4 Summary of results.....	26
4. Discussion.....	30
4.1 Pool frog phylogeny.....	30
4.2 Climatic niches of northern and mainland pool frogs.....	32
4.3 Summary and conclusions.....	34
References.....	35
Appendices.....	40

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1. Introduction

Biodiversity is decreasing at rates equivalent to historic mass extinctions, and is largely driven by increasing anthropogenic pressure on our planet (Koh *et al* 2004). This has immense implications for the stability of almost all ecosystems, which generally rely on a diversity of interacting organisms to maintain their natural balance. We tend to look at diversity in the most obvious subdivisions, such species, but often overlook what lies between these lines (Landres *et al.* 1999, Goodman 1975). With the dawn of genomic techniques and powerful computer analysis, science has opened new avenues to study diversity outside of conventional categories (Coates *et al.* 2018). This has allowed us to study cryptic units of diversity such as subspecies or hybrids. This diversity, that crosses the conventional units of species, can allow greater genetic exchange and diversity and facilitate local adaptation (Arioli *et al* 2010, Charney 2012), leading to more robust populations and ecosystems. Furthermore, hybrid systems can also lead to the species complexes which support larger systems such as superspecies and ring species (Amadon 1966, Irwin *et al* 2001). These complex systems present a challenge for conservation, and it is important to consider the implications of these interactions when deciding where conservation efforts should be targeted.

One key requirement in conservation efforts is the suitability of climate, and how this may vary across a species complex. With anthropogenic climate change shifting climates globally, information about the historic and present climate preferences of a species is vital in forming dynamic conservation efforts to understand declines and plan for the future. This is particularly poignant when these efforts are aimed at reintroducing the species into an area of its historic range, as climate change may mean this area is no longer suitable, and may even have driven the local extinction in the first place, but also open up new suitable areas where conservation efforts may be more effective (Griffiths and Pavajeau 2008).

Pool frogs (*Pelophylax lessonae*) present an interesting opportunity to investigate the complexities of such intraspecific diversity in a conservation setting. They are small (~8cm snout to vent), common water frogs that inhabits still or slow flowing water bodies across much of Europe, and exhibit extremely high diversity genetically, morphologically and behaviourally across this range (Wycherly *et al* 2002, Zeisset and Hoogersteger 2018, Arioli *et al* 2010). Pool frogs were initially thought to be relatively recently introduced to Britain from southern and central Europe through anthropogenic means, with several UK populations having documented introduction routes in the 19th and 20th centuries (Gleed-Owen 2000). They were therefore treated as an invasive species and were not targeted for conservation (Beebee *et al* 2005). However, with widespread historic reports of the species in eastern England, several lines of research suggested that they were, in fact, native. First, pre-fossil remains of the species from ~500AD were discovered in Lincolnshire and possibly Norfolk, which predated known anthropogenic introductions (Gleed Owen 2000). Second, a population of pool frogs in Thompson Common in East Anglia were found to look and behave differently to other known UK populations of pool frog (Beebee *et al* 2005) as well as possessing unique bioacoustics (Wycherly, Doran and Beebee 2002), and distinct genetics (Zeisset and Beebee 2001). Together, this evidence suggested that these were a relict population, not originating from modern introductions from southern or central Europe. Unfortunately, this information came too late, with the last pool frog being caught at Thompson common in 1986 and no more being seen

since then. Since the mid-1990s, Natural England have been running the Pool Frog Recovery Programme, which has reintroduced pool frogs to Thompson Common. The source population for the reintroduction was of Swedish descent, as genetic analyses suggested that the relict population on Thompson Common were most similar to Swedish and Norwegian pool frogs (Snell *et al* 2005). Now, 30 years later, this programme is looking to expand and so requires information into the ecology of these frogs to inform future conservation (Baker 2018).

Given the close geographic proximity of England to mainland European countries such as France and Belgium, it was somewhat surprising that native UK pool frogs were found to be genetically closest to frogs from Scandinavia, which appeared to form a distinct genetic group consisting of small, isolated populations of dark-coloured frogs in Scandinavia, Estonia and Britain, known as northern pool frogs, or the northern clade (Snell *et al* 2005, Zeisset and Beebee 2002). It has therefore been proposed that these populations are relicts from the early Holocene, when the species had colonised northern latitudes, before being isolated by melting glaciers raising sea levels in the post glacial period around 9,500 years ago (Snell *et al* 2005, Zeisset and Beebee 2002, Zeisset and Hoogesteger 2018). However, current phylogenetic work has not created a rooted phylogeny, which makes it difficult to interpret the level of genetic divergence between the northern clade and other groups, as well as making a directional colonisation route tricky to infer. Studies found the genetic diversity of these populations to be drastically lower than those in mainland Europe, possibly resulting from repeated years of mass mortalities due to harsh winters (Sjögren 1991, Snell *et al* 2005). However, they also suggested that the “northern clade”, as they have been named, have evolved plasticity in their thermal tolerances during development when compared to continental pool frogs, likely because of the evolutionary pressure induced by the northern climate (Orizaola and Laurila 2009, 2016). These populations, aside from the already extinct British population, have been facing declines, with the Norwegian population being limited to just 4 ponds, and reports of chytridiomycosis in Sweden. This raises the need for a better understanding of the ecology of pool frogs, and the northern clade in particular, and placing urgency on its regeneration in England (Kärvemo *et al* 2020, Baker 2018).

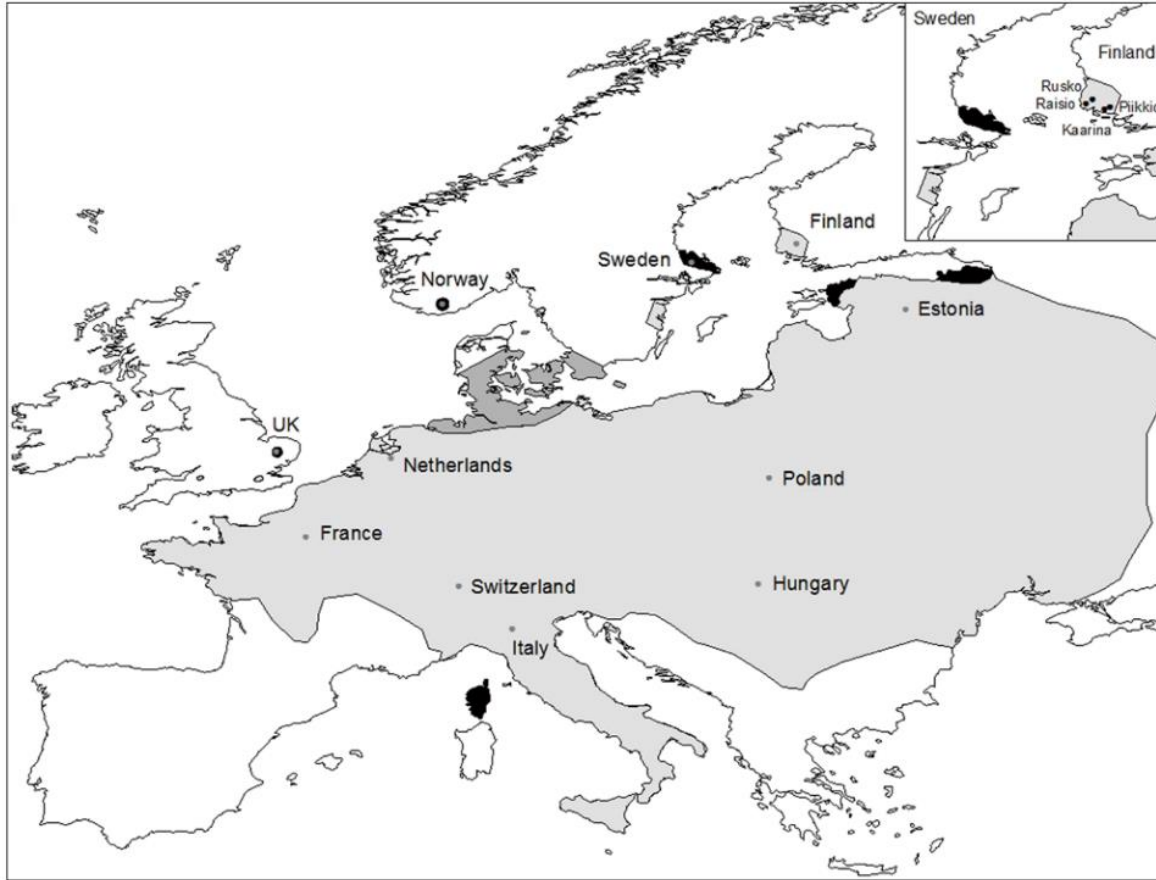


Figure 1. The distribution of pool frogs (*Pelophylax lessonae*) from Zeisset and Hoogersteger (2018), showing the populations existing in a klepton complex in grey, and populations of only pool frogs in black. The place names represent samples taken for the genetic analysis in Zeisset and Hoogersteger (2018).

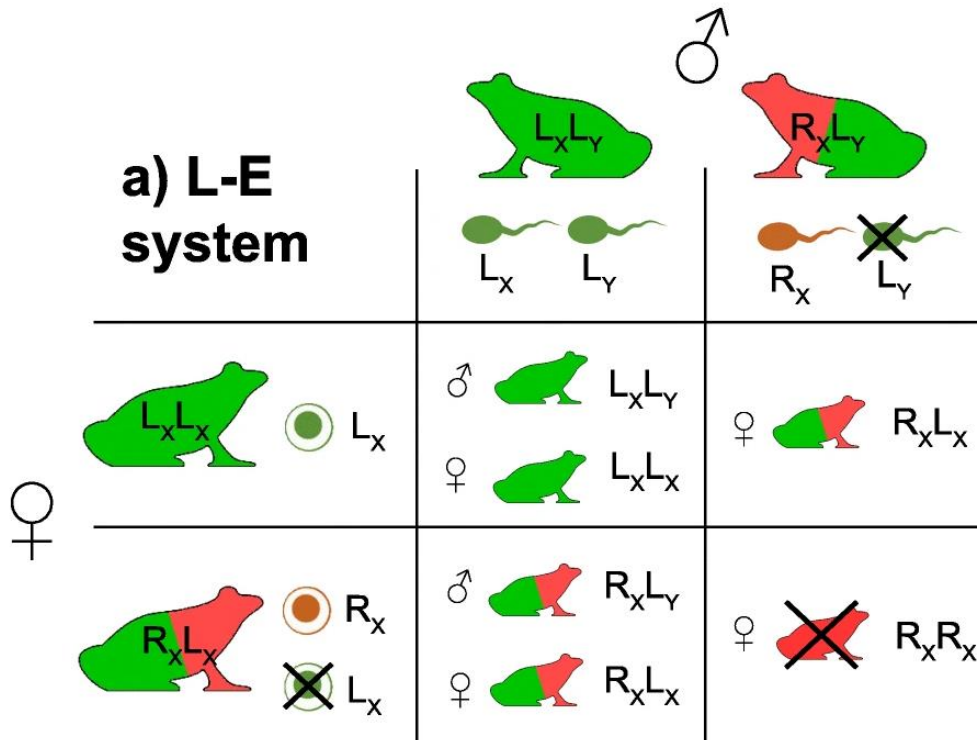


Figure 2. The “L-E” *Pelophylax* complex gamete inheritance system as shown in Dubey *et al* (2019). Pure *P. lessonae* labelled “ $L_x L_y$ ” or “ $L_x L_x$ ” naturally breed with cooccurring kleptons, *P. kl. esculentus* (RL), which themselves are a result of hybridisation between *P. lessonae* (LL), and *P. ridibundus* (RR- not shown in this figure). Note that L_x is conserved across kleptogenesis.

Pool frogs on the continental mainland exist in stable, kleptogenetic complexes, where a pool frog can breed with a marsh frog (*P. ridibundus*), producing the klepton edible frog (*P.kl. esculenta*), which can further interbreed with both species (figure 2) (Dubey *et al.* 2019, Leuenberger *et al* 2014). All three of these frogs exist naturally in a kleptogenetic system, but over time the pool frog is sometimes outbred and outcompeted from these systems (figure 2, Christiansen 2009, Holsbeek and Joris 2010, Dubey 2014). However, northern clade populations, along with another population on Corsica are not known to naturally co-occur with other *Pelophylax* species, and so do not naturally experience this phenomenon, being the only cases of this for the species (Zeisset and Hoogesteger 2018). The possibility of anthropogenic hybridisation of the pool frog in England has recently become a concern due to the presence of invasive marsh frogs in the country (Pille *et al* 2024, Holsbeek and Joris 2010, Mackay 2018). These frogs are often accidentally transported in water plant or fish trade and can rapidly become established due to their high fecundity and tadpole behaviour (Beebee and Griffiths 2000, Mackay 2018). As northern pool frog populations do not naturally occur alongside the klepton complex, understanding how their ecology is different from frogs that exist within the klepton complex could assist in defending the species from the threats posed by invasive populations of marsh or edible frogs.

The objective of this study is to further our understanding of the conservation of the pool frog in the UK. To achieve this, I first investigate the degree of divergence between the northern clade of pool frogs and mainland populations by using mitochondrial DNA (mtDNA) sequences to build a rooted phylogeny. This also provides insights into how the species colonised the colder areas of northern Europe forming the distribution seen today. My tree builds on previous genetic analyses of pool frogs (Zeisset and Hoogersteger 2018, Snell *et al* 2005) by including additional sequence data derived since these studies were conducted, and through rooting the tree using a closely related species, which allows us to identify clades. Secondly, I model the climatic niche of pool frogs in northern Europe, with a focus on understanding potential ecological differences between the northern clade populations and their mainland counterparts and predicting the suitability of the UK and Scandinavian climate for future reintroductions.

Study Predictions:

Northern clade populations likely became genetically isolated after a period of glaciation, with the British population likely being isolated around the time sea levels rose to cover the Dogger land bridge around 10,000 years ago (Snell *et al* 2005, Zeisset and Hoogersteger 2018). Pool frogs are generally a widespread and diverse species, and so I predict that their climatic niche will be broad, meaning most of Europe will provide a suitable climate (Zeisset and Hoogersteger 2018, Arioli *et al* 2010). However, northern clade frogs will likely have a narrower climatic niche, possibly due to the selective pressure applied by the cold climate (Sjögren 1991). The climate niche of northern clade and mainland frogs will likely be similar, with northern frog's niche fitting inside the larger mainland niche, likely being limited to warmer areas due to the adverse temperatures of northern latitudes (Orizaola and Laurila 2009, 2016). The niche modelled from mainland occurrences will be widespread within England, although likely not the northern parts of Scandinavia. Northern pool frog niche will likely pick out warmer and wetter parts of both Britain and Scandinavia as being more suitable.

2.Methods

This study takes two approaches. The first used cytochrome B sequences previously uploaded to GenBank to estimate a phylogeny, with tree tips showing the geographic location of each sample, which was then rooted. This informs us on the phylogeography of the pool frog, including the colonisation paths, the relative divergence of northern clade populations and inferring when kleptogenesis may have begun in this species complex. The second method investigated the climatic niche of pool frogs across Europe, to identify what climatic factors most influence their distribution. I then subsetted data of northern clade populations and compared the results to mainland populations occurring nearby to identify any features that vary between them. Finally, I used the best fitted models generated to predict suitable habitat across Europe, with a focus on how the models predict the distribution of suitable climates across the Baltic area, Scandinavia and England, which may reveal places where undiscovered populations may exist, and where conservation efforts may be best placed.

2.1. Phylogenetic tree estimation

Data collection: The GenBank accession numbers and geographic localities of sequences used for phylogenetic tree estimation are shown in Appendix A. This was a collection of 50 georeferenced Cytochrome B (CytB) mitochondrial sequences of at least 250bp, from a range of locations within Europe that could be used to infer a colonisation path for the species. The *P. lessonae* mitochondrial genome is generally conserved across the *Pelophylax* hybrids (*figure 2*) (Hoffman *et al* 2012, Dubey *et al* 2019). This means our samples, which will include kleptons (*P. kl. esculenta*), likely have CytB genomes originating from *P. lessonae*, and so using CytB we can detect pool frog lineages even when hybridised. This is appropriate for this study as the mainland populations by definition are hybrid populations and so it is appropriate to include these sequences. CytB is a commonly used marker for phylogenetic reconstruction and determining divergence within genera due to its relatively high sequence variability. The highly variable nature of these sequences allows for differences between closely related taxa, such as within a single species to be inferred (Castresana 2001). This is especially important for northern frogs which have notably low genetic diversity. To root the tree, I assembled 20 CytB sequences from *P. perezi*, a species that diverged from pool frogs around 20mya and is not involved in natural hybridisation with the species due to being largely restricted to the Iberian peninsula. (IUCN 2024). The sequences were aligned by codon using MUSCLE (Multiple Sequence Comparison by Log-Expectation) and similar sequences were clustered via UGMA (Unweighted Pair Group Method with Arithmetic Mean) on MEGA X, from which a more refined tree can be inferred (Kumar *et al* 2018). Due to many samples being incomplete, gaps were removed by pairwise deletion for analysis.

Phylogenetic Inference: The phylogeny was inferred using the Maximum likelihood method, with Hasegawa-Kishino-Yano model with gamma distributed rate heterogeneity (6 categories), and 1000 bootstraps, to model the differences in rates of base substitution among sites (Hasegawa *et al* 1985). Model selection was based on the statistics shown in Appendix B, using BIC and AIC values to weigh up the fit of each model. The initial tree for the heuristic search was obtained by Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances, which was estimated using Maximum Composite Likelihood (MCL) approach. This analysis involved 70 nucleotide sequences, ranging from 275bp to 1143 bp. This analysis was conducted in MEGA X (Kumar *et al* 2018).

2.2. Climatic niche modelling

This climatic niche modelling study uses Species Distribution Models (SDMs), which analyse if patterns in environmental variables, such as climate, appear to predict the presence or absence of a species across their known range. This is done by creating a dataframe of species presence/absence, and environmental factors, and associating it with a spatial raster grid of a geographic area. This creates a grid of squares that each have a list of environmental statistics, as well as a binomial species presence/absence. As ecological relationships are notoriously stochastic, and often have complex non-linear or interactive relationships, I compare two methods for fitting the relationship between temperature and precipitation climate, as a predictor for pool frogs' occurrence. These methods are compared and assessed for fit (Table 1), to model the climatic preferences of the species (figures 6 and 7), compare niche variety within the species (Table 2), and predict the distribution of suitable climates for pool frogs across northern Europe (figures 8,9 and 10).

Data collection and selection of environmental variables:

The environmental variables included in the climatic modelling were based on our prior knowledge of pool frog ecology. Temperature (and how it changes seasonally) is likely to be important in determining pool frog distributions as these diurnal frogs breed seasonally from March to June and often bask on sunny banks during the summer. Spawn is laid in small clusters, usually amongst aquatic vegetation, and tadpoles usually metamorphose from July to August, preferring breeding season temperatures of around 23°C (minimum approximately 16°C) (Michaels and Försäter 2017). Developed frogs hibernate over the colder months, from late September to May (Sjögren *et al* 1988, Tatterall and Ultsch 2008), usually in holes or burrows, although occasionally aquatically (Holenweg and Reyer 2000, Sjögren-Gulve 1998). Precipitation is also likely to be important as the availability of water bodies is essential for their reproduction and dried up banks are known to provide poor habitat for basking adults (Baker 2023). Finally, altitude, which aside from influencing temperatures and precipitation, is known to heavily influence the habitat preferences of many amphibian species (Lyons and Arbuckle 2024). High elevation habitats can often be unsuitable due to exposed conditions creating low humidity, which can limit moisture dependant terrestrial species (Baken *et al* 2021). The steep topology of high altitude environments can also equate to a lack of still water bodies, an essential part of pool frog ecology, and heavy rain in such conditions is known to wash away aquatic amphibians that are not adapted to such habitats (Blaustein *et al* 2010, Bickford *et al* 2010, Michaels and Försäter 2017). Furthermore, precipitation is more likely to fall as snow at higher altitudes, which therefore may strongly affect the way precipitation influences the distribution of suitable habitat and so may create an important interaction between these two variables in the models.

Environmental data was downloaded from www.worldclim.org for the following variables at a resolution of 0.5 minutes of a degree (Fick and Hijmans 2017):

- Mean annual temperature
- Temperature Seasonality (standard deviation $\times 100$)
- Mean Temperature of Wettest Yearly Quarter
- Mean Temperature of Driest Yearly Quarter
- Mean Temperature of Warmest Yearly Quarter
- Mean Temperature of Coldest Yearly Quarter
- Annual Precipitation
- Precipitation Seasonality (Coefficient of Variation)
- Precipitation of Warmest Yearly Quarter
- Precipitation of Coldest Yearly Quarter

Altitude data for Europe was downloaded using the `get_elev_raster()` function in the `elevatr` package (Hollister and Shah 2023).

Occurrence data for pool frogs was accessed from gbif.org (accessed 7th January 2025) (<https://doi.org/10.15468/dl.hrvn9p>) and consisted of all records for the species minus occurrences in Britain and Spain, as all of these occurrences stemmed from introductions (IUCN 2024).

As across mainland Europe, pool frogs only occur within hybrid systems, it is poignant to think of the “northern clade” populations as the only places we can be relatively certain the data only contains pool frogs (alongside Corsica). To investigate potential differences in climatic niche between northern and mainland pool frogs, I first ran models containing all occurrences of pool frogs (both northern and mainland). I then investigated the northern clade distribution separately by using data only from the areas stated by Zeisset and Hoogersteger (2018) consisting of East Anglia-England, Uppsala-Sweden, Turku-Finland, Arendale-Norway, northwest Estonia and St Petersburg-Russia (<https://doi.org/10.15468/dl.bt7vfp>). In this dataset, I took a generous approach to assigning records to the northern clade populations stated in Zeisset and Hoogersteger (2018), as the data for northern clade populations is inherently limited by their small distribution and the methods used in the SDMs require large datasets for robust results. Northern pool frog data was subset from the full occurrence data along geographic lines where there is a gap in occurrence points, so that pseudo-absence points are not generated in areas where occurrences have been excluded. I also filtered occurrence points to 1km accuracy in the full dataset, and 2.5km in the northern dataset. Occurrences within 500m of a country centroid were also removed to further filter inaccurate coordinates. The sparse northern data may lead to overfitting in the models as occurrences appear to be very geographically limited across the study area, and so may not occur at points where truly optimal conditions are. Furthermore, the edge of range nature of the northern study area will mean models will likely be more sensitive to the negative effects of adverse conditions, rather than the positive effects of optimal ones.

Data preparation:

Pool frog distribution data was uploaded to R studio v4.2.3 as a list of latitude and longitude per presence record of the species. This list was then converted to a raster using the raster package v3.6 (Hijman 2023) and plotted over a map of Europe (Figure 3). The raster resolution was set so each square is 0.5 latitude and longitude and multiple record points in each square were removed. A polygon of 50km radius was then created around the occurrence points to create a sampling area with which to create randomized pseudo-absence points. This gave presence and pseudo-absence data in the locality of recorded occurrence. Northern clade pool frogs had 490 presence points and 90 pseudo-absence points, and the full distribution had 13461 presence points and 1046 pseudo-absence points.

The climatic datasets were uploaded to R studio using the geodata package v0.4-7 (Hijmans 2023). These were then cropped to fit over the extent of the pool frog distribution, plus longitude and latitude degrees further (Figure 4). This then was combined into one dataset of both climate variables, altitude, and occurrence data for each raster square, from which I trained the models.

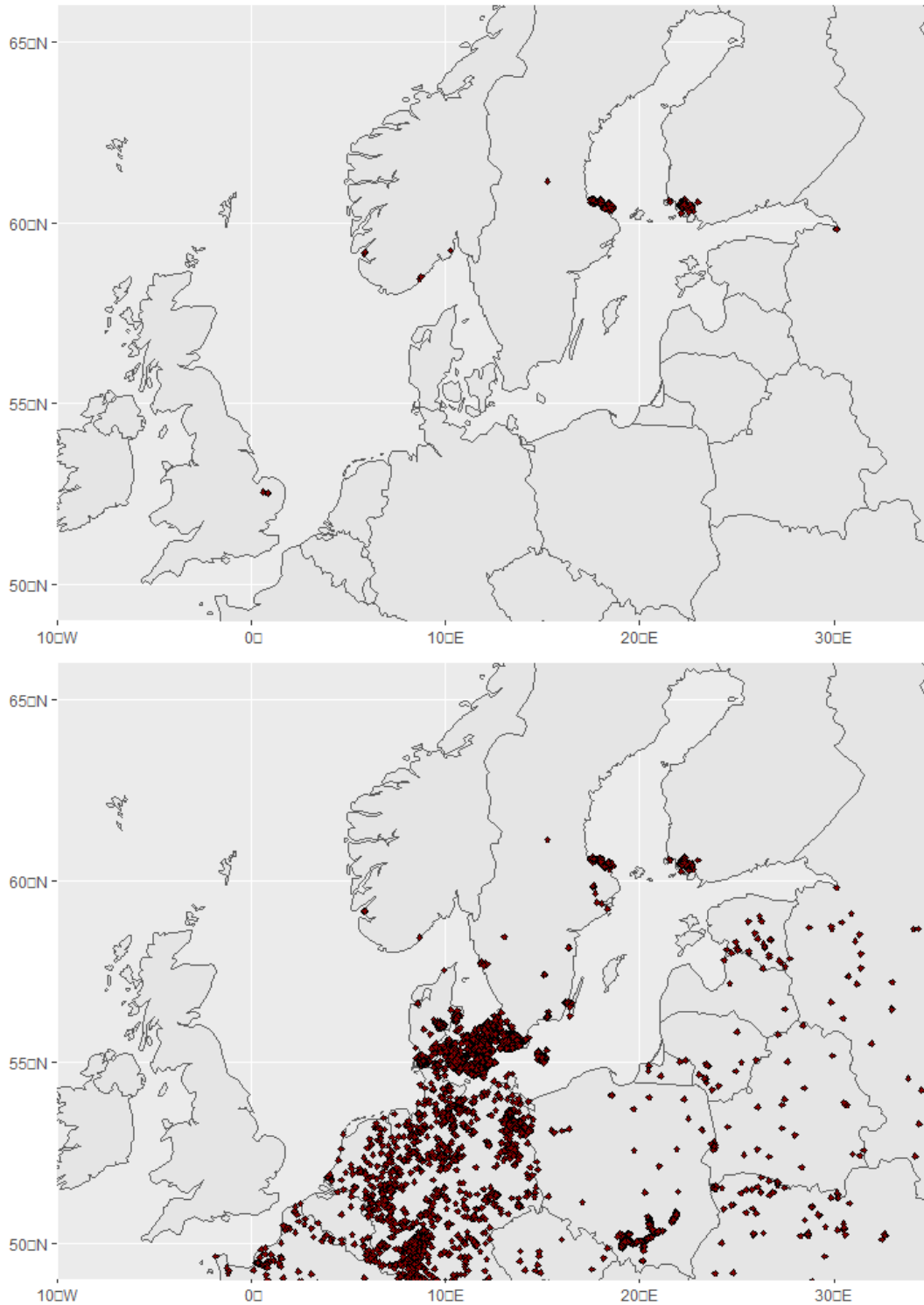


Figure 3: The occurrence data used for species distribution modelling mapped onto northern Europe. Above: Northern population occurrence points. Below: All pool frog occurrence points minus introduction in Britain and Spain.

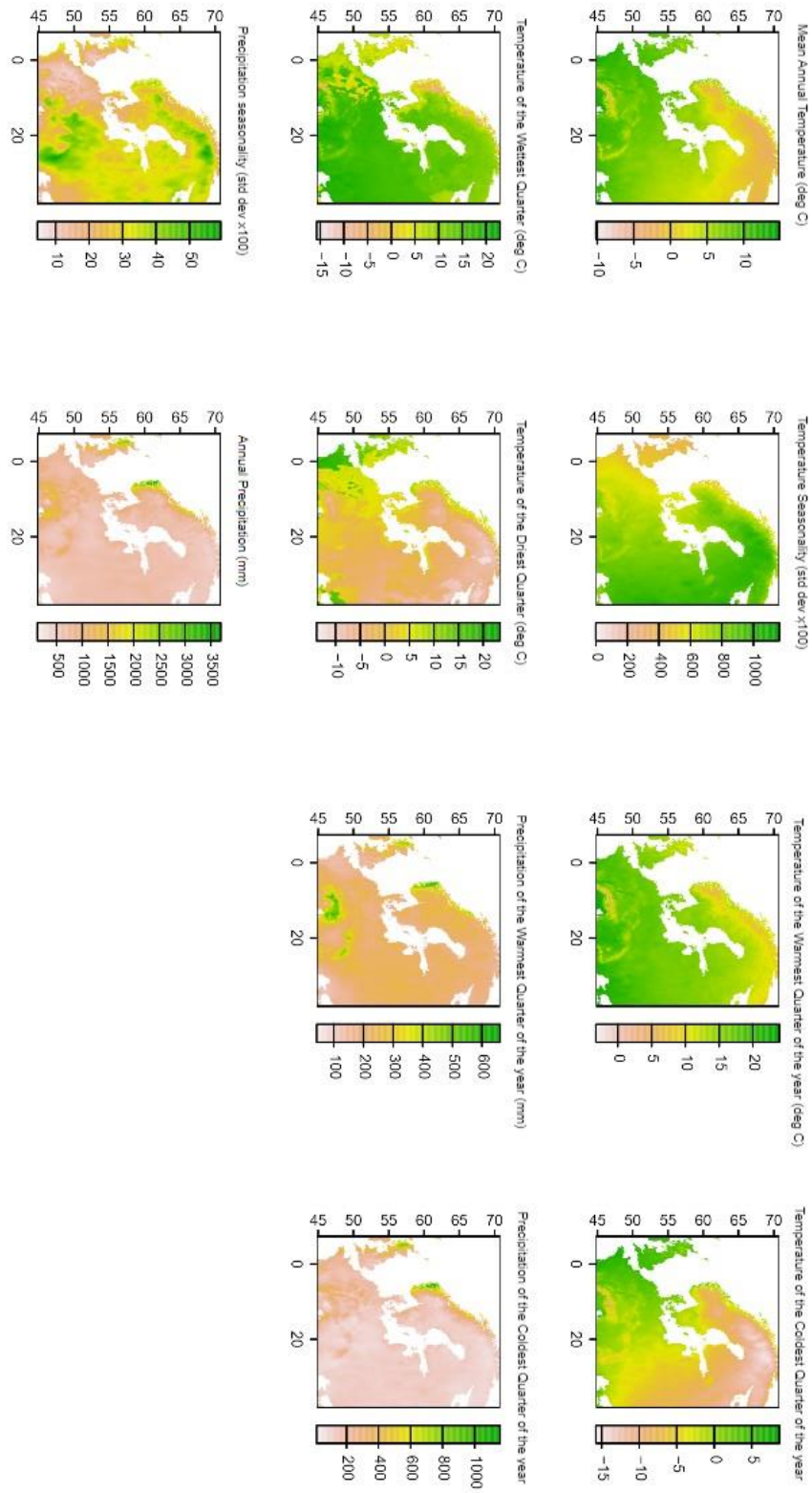


Figure 4: The climatic variable data used in species distribution modelling mapped onto Europe.

Models:

The models used to predict pool frog occurrence were based around 3 hypotheses, and are as follows.

- Model 1. Temperature: This model uses the following predictor variables; mean annual temperature, mean temperature of the hottest quarter, mean temperature of the coldest quarter, temperature seasonality (standard deviation $\times 100$), and a control for altitude included. This aims to specifically test the effect of temperature conditions on occurrence.
- Model 2. Precipitation: This model uses the following predictors: mean annual precipitation, mean precipitation of the warmest quarter, mean precipitation of the coldest quarter, and a control for altitude. This aims to specifically test the effect of precipitation conditions on occurrence.
- Model 3. Temperature-precipitation combined: This model uses the top three most influential variables (tested using the BRT model described below) from the previous two models to create a more tailored model that in theory with encompass the information tested previously to give a better fitting model.

Model justification and performance

The three models were tested using Boosted Regression Trees (BRT) a machine learning method which is widely used for predicting species distribution (Zurell 2020), and two statistical methods; General Additive Models (GAMs) which are a common way of modelling non-linear relationships in ecology, and a simpler General Linear Models (GLM). These methods were chosen to consider whether the relationship between occurrence and climate are simply linear, non-linear but related, or more complex and interacting. The models were assessed using the sdm package in R studio v1.2-46 (Naimi and Araújo 2016).

Model fit was assessed using the following statistics (Table 1). Area under the curve (AUC) represents the ability of the model to predict occurrence on unseen data points, and so is especially useful when using the models to predict suitable climates in areas with no occurrence data. An AUC of 0.5 means the model fits as well as random guessing, and AUC of 1 means the model perfectly predicts unseen data. Correlation (Cor) indicates the predictive ability of the climate variables in the model, with 0 meaning the variables have no influence on predicting occurrence and 1 meaning the variables perfectly predict occurrence. True skill statistic (TSS) also assesses the predictive ability of the model by comparing true and predicted species distribution, with 0 having the predictive power of random guessing and 1 being a perfect fit. Deviance (Dev) of the model predictions from the true data, with lower deviance meaning the model predictions fit more of the true occurrence data.

Mapping predicted climate suitability:

The models, after being trained on the known occurrence data, were used to predict the likelihood of occurrence across the rest of northern Europe, using the raster containing climate data. This uses the learnt patterns in variables to assign each raster grid a suitability score, which represents how well its climate matches with what the model has learnt. A high suitability means the model has matched the climate as a fit to those with known species presence points.

2.3 Niche similarity calculations:

Niche similarity compared northern data to a subset of the full distribution dataset, which was cropped to -5 to 30 degrees longitude and 55 to 66 degrees latitude, and with all points that occur in the northern clade data removed. Niches were modelled using the 3 BRT models for both occurrence datasets. I then compared the overlap of each model between the two datasets using the niche similarity function in the *dismo* package v1.3-9 (Hijmans *et al* 2023). I used four statistics to quantify the similarity between northern and mainland climate niche. These were; Schoeners D statistic which indicates the proportion of niche overlap, Hellinger Distance which compares the two niches and quantifies the differences between them, Corrected Hellinger Distance which works in a similar way but corrects for the uneven sample sizes, and Bray-Curtis Dissimilarity which quantifies inconsistencies between the niches (Rödder *et al* 2011). This will allow us to further assess if northern frogs vary significantly in their niche from their mainland counterparts. These analyses compare the resulting niches in the same space (ie northern Europe) as opposed to the models on their own which only test the influence local conditions on local occurrences and do not directly compare the two niches directly.

3. Results

3.1 Phylogenetic Reconstruction

I found generally low phylogenetic structure across the range of pool frogs based on CytB sequences (Figure 5). Specifically, I recovered only three clades within the tree, two of which comprising only a single sequence each. The earliest diverging pool frog sequence in my tree is one of the two Italian sequences in my dataset, followed by a split between the single Danish sequence and an undifferentiated clade containing the other 48 of the 50 *P. lessonae* sequences. Notably, the Danish sequence is inferred to be almost identical to the ancestor of all pool frogs in the sample except for the basal-most Italian sequence.

3.2 Climate Niche Models

Model performance comparisons revealed that the BRT models consistently outperformed the GLMs and GAMs (Table 1). Area under the curve (AUC) values, correlation values, and true skill statistic (TSS) were higher for all BRT models in comparison to the GLM version of the same model (Table 1). GAMs also produced relatively strong AUC, Cor and TSS values for both datasets. However, deviance was lower for all the BRT models, except for the precipitation and combined models for the full pool frog distribution. GAM model resulted in very large deviance values for the northern data. The BRT models were therefore selected as the best performing and so were used for further analysis as .

Table 1. The model performance outputs from testing GLM and BRT methods.

Model-dataset	GLM (AUC, Cor, TSS, Dev)	GAM (AUC, Cor, TSS, Dev)	BRT (AUC, Cor, TSS, Dev)
Temperature-full distribution	0.61, 0.16, 0.21, 1.21	0.62, 0.2, 0.23, 1.21	0.65, 0.23, 0.26, 1.2
Precipitation-full distribution	0.61, 0.18, 0.22, 1.19	0.62, 0.19, 0.22, 1.2	0.63, 0.2, 0.22, 1.19
Combined-full distribution	0.63, 0.2, 0.24, 1.2	0.64, 0.23, 0.26, 1.2	0.65, 0.24, 0.27, 1.19
Temperature-northern clade	0.63, 0.19, 0.45, 2.28	0.59, 0.01, 0.37, 10.53	0.69, 0.21, 0.53, 0.76
Precipitation-northern clade	0.66, 0.21, 0.47, 1.65	0.7, 0.34, 0.5, 8.7	0.72, 0.32, 0.55, 0.95
Combined-northern clade	0.64, 0.18, 0.41, 1.68	0.73, 0.45, 0.59, 6.06	0.7, 0.26, 0.48, 0.96

Influence of climate variables on pool frog occurrence:

Using data from all pool frogs, there was variation in the environmental variables that the BRT models identified as most influential (Figure 6). The temperature model found that the temperature seasonality was most influential, followed by the annual average temperature, and average temperature of the coldest quarter. For the precipitation model, seasonality in precipitation stood out as being particularly important in comparison to the other variables included, especially as altitude ranked second most influential but last in the temperature model. The combined model found relatively even influences of the variables, but annual average temperature stood out as the most important.

Focussing only on the northern populations, altitude stood out as a strong influence (Figure 7), suggesting this is a good predictor for northern pool frog occurrence. In the temperature model, temperature seasonality was ranked as the second most influential after altitude. Interestingly, temperature of the coldest quarter of the year had relatively little influence in the decision making of this model, which is surprising for an ectotherm species at high latitudes. Additionally, annual

average temperature ranked the most influential in the combined model, above altitude, despite being lower in the temperature model. This may suggest that these two variables are highly interacting (ie it gets colder with higher elevation) and so the model may struggle to separate their individual effects.

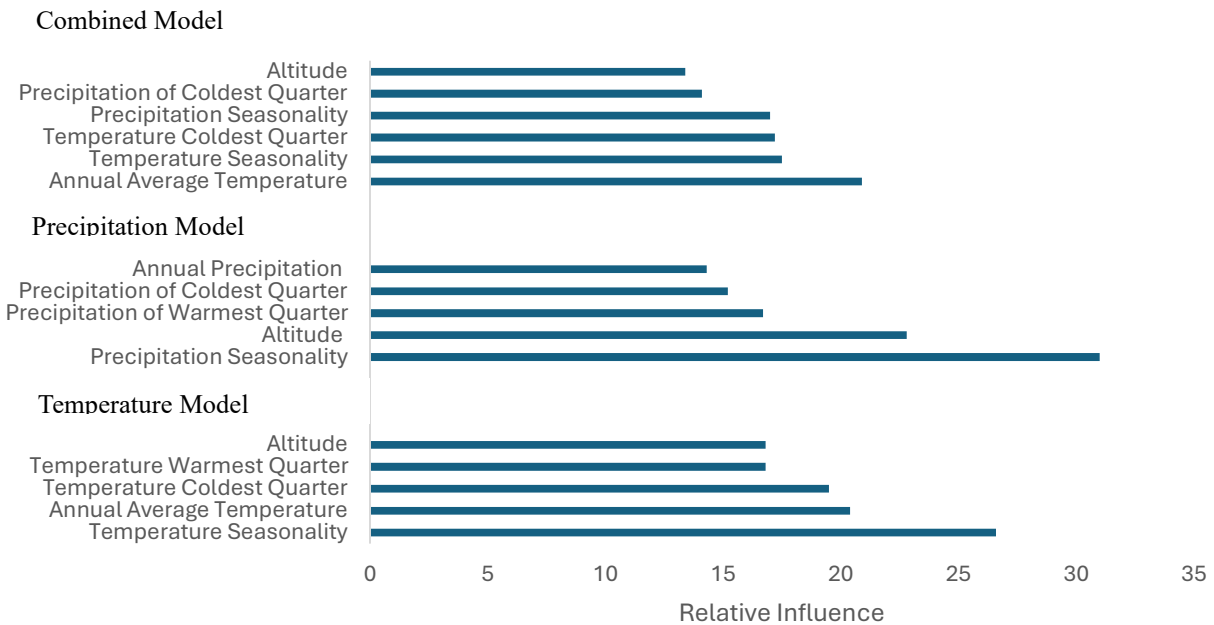


Figure 6: The relative influences of each climate variable included in the three BRT models in predicting pool frog occurrence. The x-axis represents the contribution of each variable to assigning the likely presence or likely absence of pool frogs to each grid square relative to the other factors.

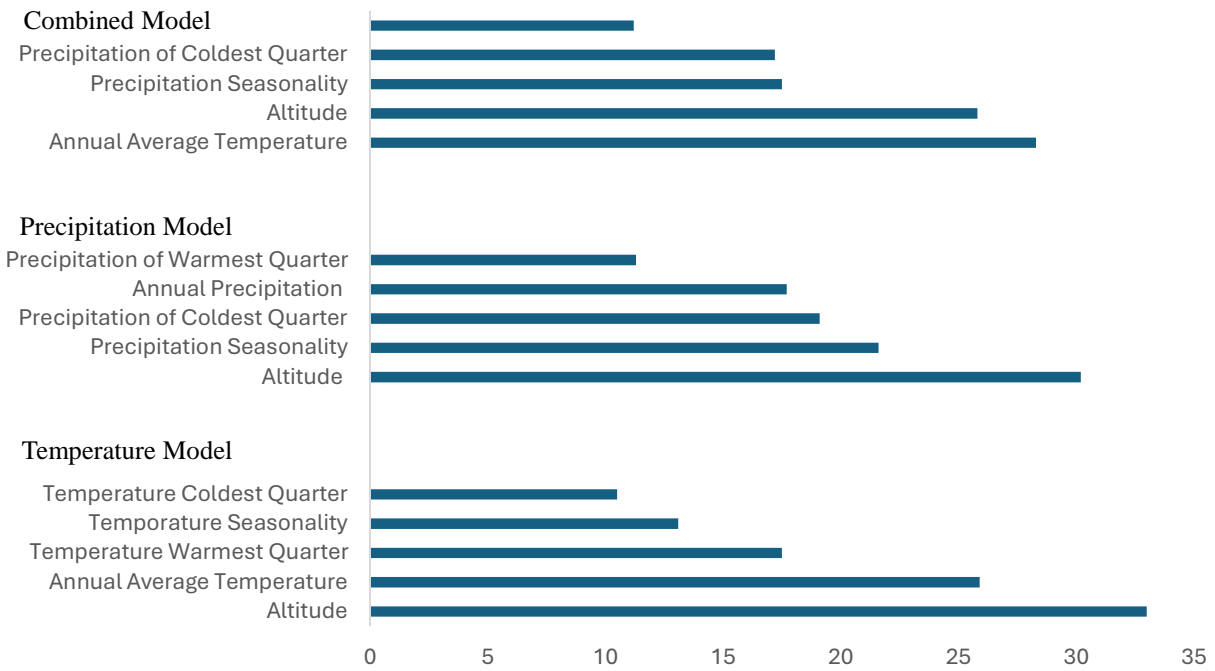


Figure 7: The relative influences of each climate variable included in the three BRT models in predicting northern pool frog occurrence. The x-axis represents the contribution of each variable to assigning the likely presence or likely absence of pool frogs to each grid square relative to the other factors.

3.3 Niche Similarity

Niche similarity indices suggest that the niches of northern frogs and mainland frogs are very similar (Table 2). The Schoeners D statistics of all three models were high, with niche overlaps of 73-79%. The Hellinger Distance (I) and the corrected Distance (ICor) infer even greater overlap, especially when accounting for the small sample size of northern frogs. The Bray-Curtis Dissimilarity (BC) suggests that there is some dissimilarity between the two modelled niches, although this statistic is based greatly around the abundance of the species in the two niches, so may be influenced by the considerable size variation between the two datasets.

Table 2: The niche similarity statistics from comparing the BRT modelled niches for the three models between mainland type pool frogs, and northern type pool frogs. Statistics are as follows: Schoeners D statistic (D), Hellinger Distance (I), corrected Distance (ICor) and Bray-Curtis Dissimilarity (BC).

Model	D	I	I Cor	BC
Temperature	0.86	0.9	0.98	0.41
Precipitation	0.89	0.91	0.98	0.43
Combined	0.87	0.9	0.98	0.42

3.4 Predictions of Suitable Habitat

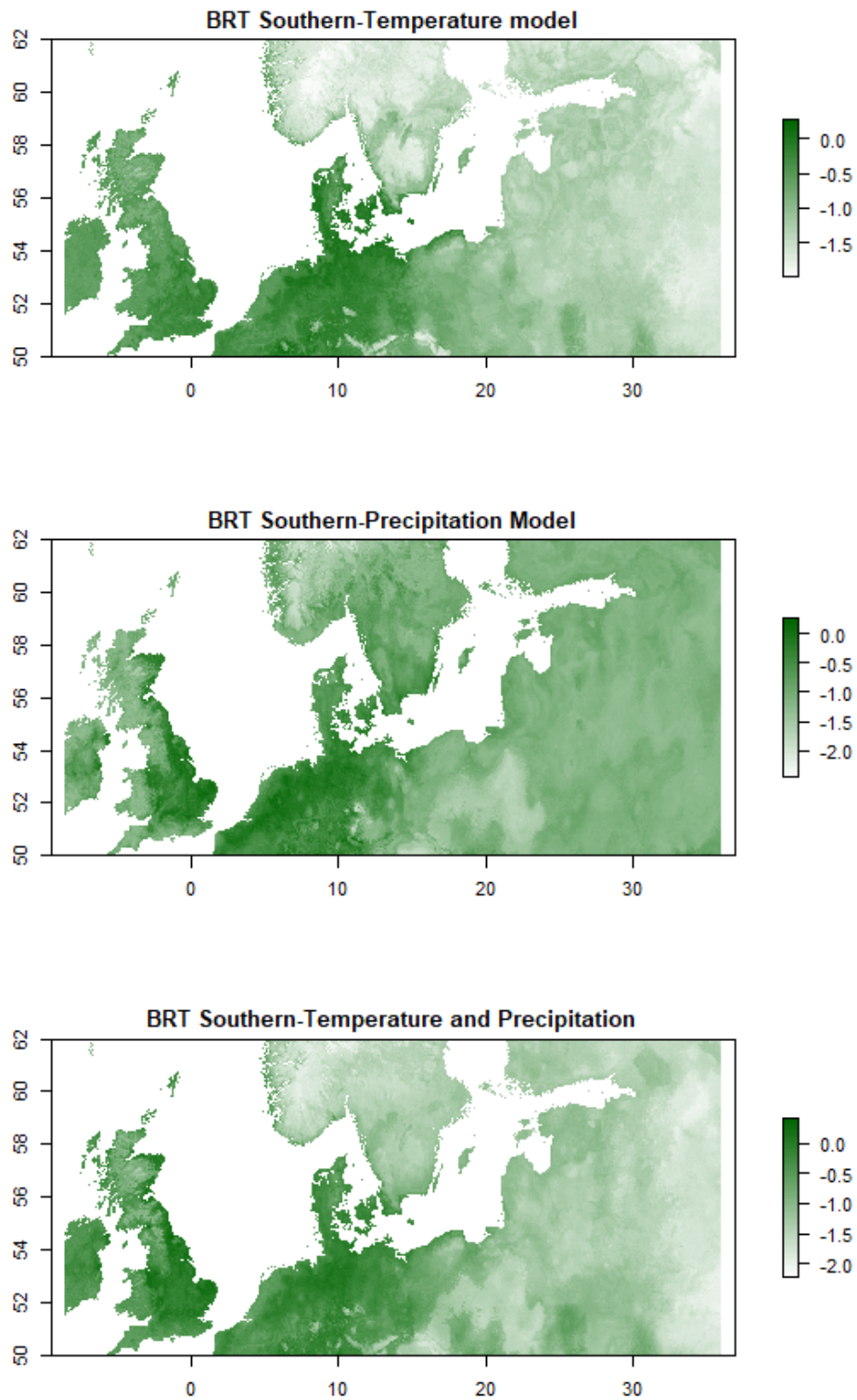


Figure 8: Suitable climate predictions for pool frogs based on the three BRT models, with blue representing relatively unsuitable areas and light green representing more suitable ones.

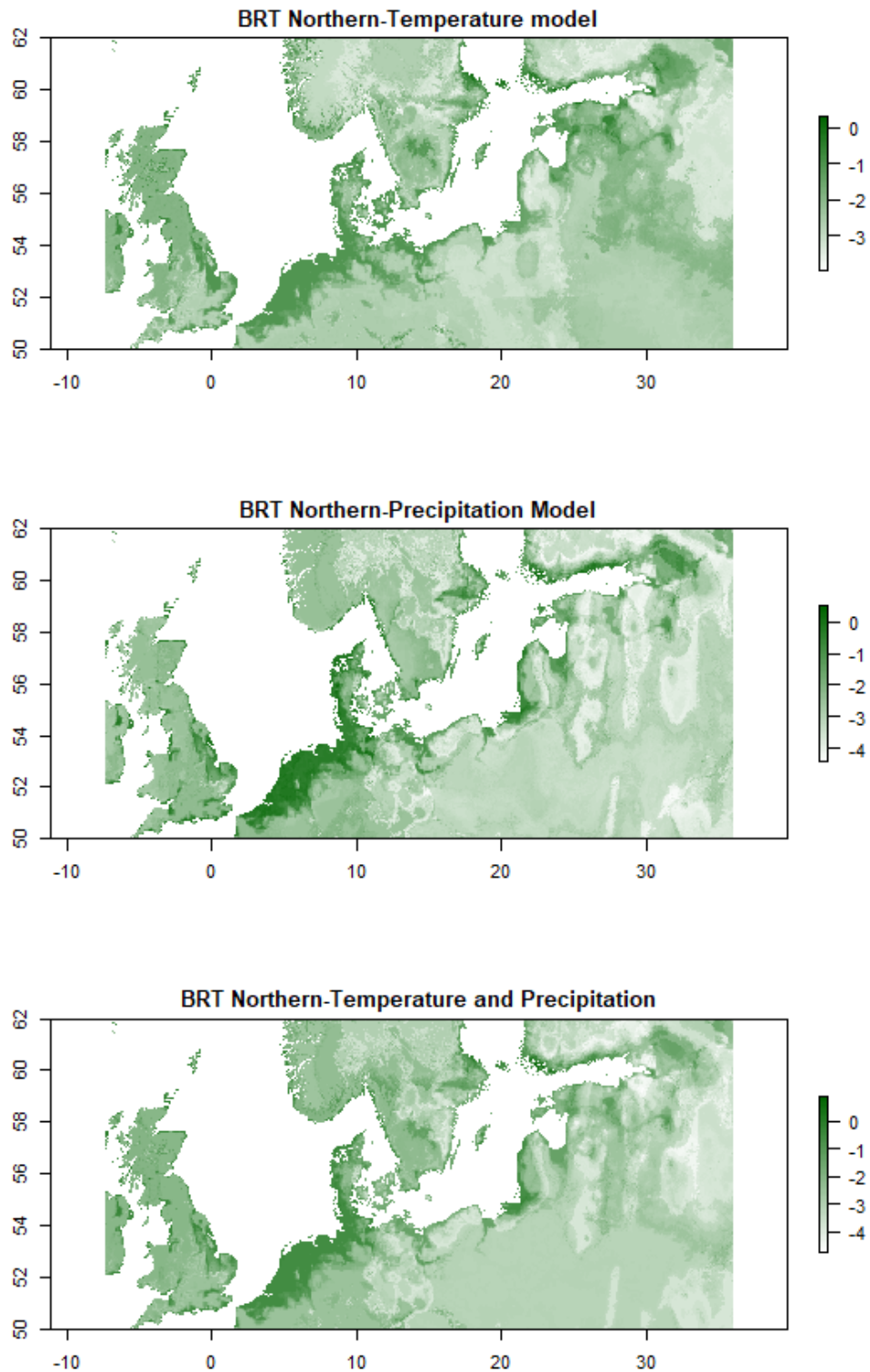


Figure 9: Suitable climate predictions for northern pool frogs based on the three BRT models, with green representing relatively unsuitable areas and light green representing more suitable ones.

Pool frogs have a wide climate niche across all three models, with only the mountainous areas of inland Scandinavia and northwestern Russia providing unsuitable temperature climate for them (Figure 8). Precipitation climates appear suitable across almost all northwest Europe, with areas of less suitability in the western and upland areas of the British Isles and Scandinavia, likely due to them experiencing heavy precipitation.

Northern pool frogs (Figure 9) have a more limited distribution of suitable climates in comparison to all pool frogs (Figure 8). Suitable temperature climates for northern pool frogs exist along low-lying, sheltered coastal areas around the North and Baltic seas, likely the warmer parts of these areas due to their exposure to the gulf stream. Inland western Europe appears slightly less suitable in all models, most likely due to the rise in elevation towards the Alpine mountain range and the upland areas of Britain, shown in more detail in Figure 10. The precipitation model also suggests unsuitable habitats in inland eastern Europe and northern Scandinavia, as well as western Scotland and Wales. This may also be due to excessive cloud cover or snowfall.

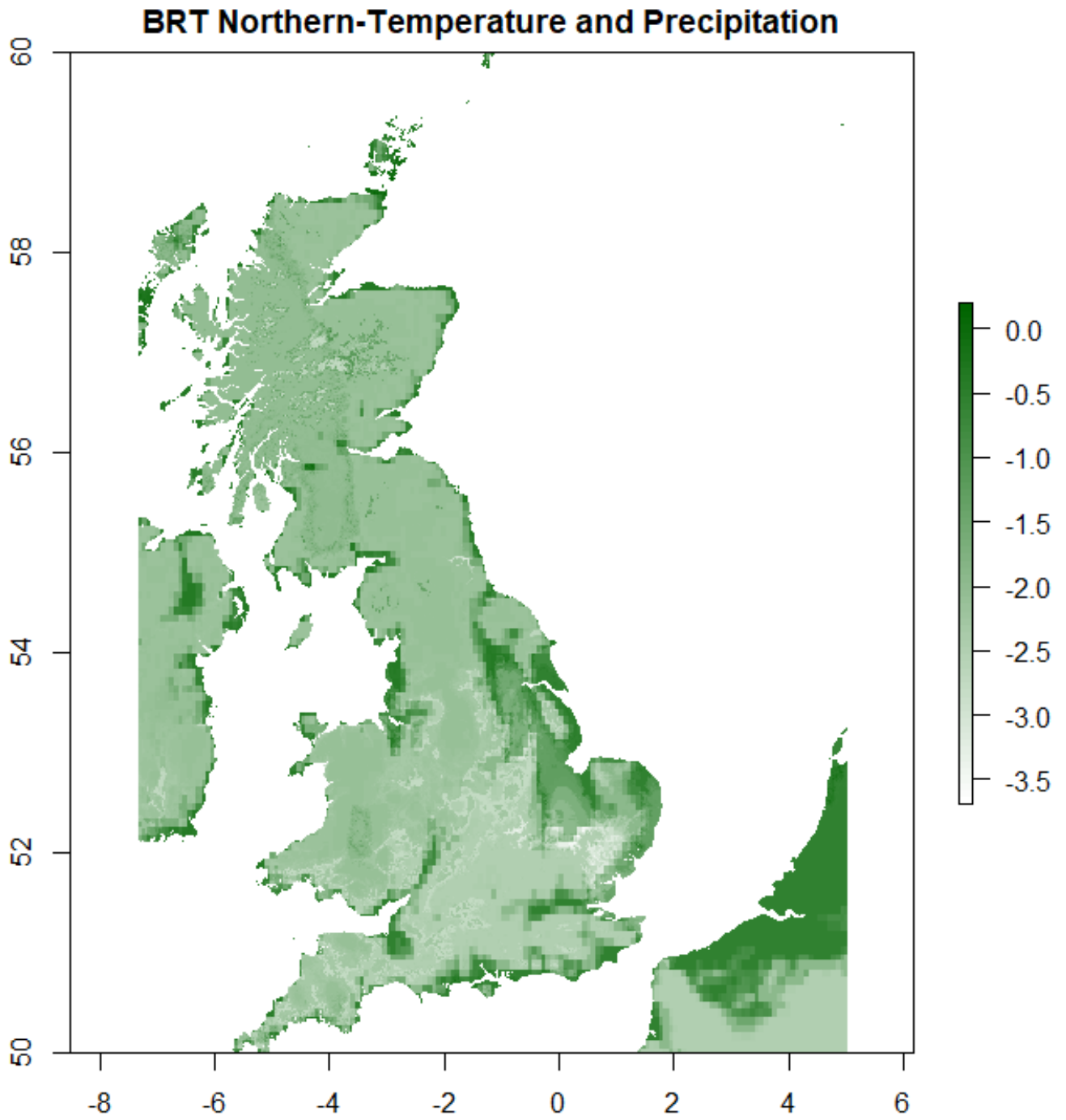


Figure 10. The predicted suitable climates mapped onto mainland Britain based on the combined model, with green representing relatively unsuitable areas and light green representing more suitable areas.

4. Discussion

4.1. Pool frog phylogeny

The phylogeny found relatively little divergence between pool frogs sampled across Europe. Nevertheless, it provided some insight into postglacial colonisation routes. Specifically, the earliest diverging pool frog sample was from Italy, supporting the idea that pool frogs used the Italian peninsula as a glacial refugium (Litvinchuk *et al* 2024, Canestrelli and Nascetti 2008). This isolation likely led to the divergence of pool frogs, marsh frogs and Perez's frog around 20mya in glacial refugia on the Italian and Iberian peninsulas and in the Balkans respectively (Dufresnes *et al* 2024). The next, and only other well supported, divergence in my phylogeny was between a sequence from Denmark and an undifferentiated clade of all other sampled pool frogs. Particularly as this Danish sequence is inferred to be very similar to the ancestral sequence of all sampled pool frogs except the aforementioned Italian sequence, this is consistent with Northward dispersal presumably to the West of the Alps, leading to the colonisation of central Europe. Note that my results are consistent with an Italy to Denmark dispersal before dispersal to other sampled areas, since a Northward dispersal to the West of the Alps would involve travelling through Germany, for which we have no samples. Following this Northward dispersal, our results suggest that pool frogs spread through the rest of Europe sufficiently rapidly that little CytB divergence occurred and no further geographic structuring is present in this marker. Future work should include multiple variable genetic markers such as ND4 or ND2 sequences, which in addition to CytB may reveal structure that this single sequence phylogeny cannot (Sjögren 1991, Hoffman *et al* 2012).

Whilst the phylogeny supported previously proposed post-glacial colonisation routes out of Italy, it did not provide any evidence for the proposed colonisation route from eastern Europe, through Scandinavia, to Britain, as samples from these locations clustered with samples from southern and central Europe with no evident geographic structuring of populations. Unlike previous studies, I found little evidence for structure between eastern and western clades (Snell *et al* 2005, Zeisset and Beebee 2001), and also little evidence of a genetically distinct clade occupying northern latitudes. There are several possibilities which might explain the lack of distinct clades in the phylogeny corresponding to geographic location. First, it appears that CytB is relatively conserved across pool frogs, with very little diversity between most of our sequences. This gene appears to be consistently derived from *P. lessonae* across the hybrid complex, suggesting that the diversity introduced into the genome by kleptogenesis does not heavily affect mtDNA, thus explaining its relatively low diversity (Hoffman *et al* 2012). Furthermore, previous studies using microsatellite markers found that populations across the north of the range (specifically the remaining native English pool frog and Norwegian and Swedish populations) were fixed for the same alleles, strongly suggesting that they are closely related (Zeisset and Hoogerstege 2018, Zeisset and Beebee 2001). Future studies incorporating a range of different sequences, such as ribosomal 16S, or more powerful analysis such as Bayesian methods may be able to further resolve this phylogeny

by allowing prior knowledge from previous studies on pool frog phylogeny to be incorporated and by testing a wider variety of possible trees which may be missed in maximum-likelihood methods which converge to a single solution. Such approaches will be aided by the development of a reference genome for pool frogs, which is currently in the process of being sequenced by the Darwin Tree of Life Project (www.darwintreeoflife.org). This can facilitate analysis of the wider genome which may highlight more useful markers in the future.

A second possibility potentially explaining the lack of geographic clades in the phylogeny relates to the origin of the samples. Whilst the geographic locations of the pool frogs sampled were known, it is possible that some of these individuals derived from introductions from other parts of their range, rather than being ancestral to the area, potentially scrambling any phylogenetic signal. Sources of translocations range from historic transport for food, to the modern pet trade and highlights the need for modern genetic surveillance to detect new routes of these cryptic invasions which can lead to outbreeding consequences and reductions in overall intraspecific diversity (Gleed-Owen 2000, Dufresnes *et al* 2020). However, since my results suggest widespread lack of structure rather than a few ‘misplaced’ sequences in an otherwise well-structured phylogeny, such introductions would have to be highly prevalent in the samples to be a plausible explanation. Moreover, the lack of any phylogenetic structure in the majority of pool frog samples (whether geographically clustered or not) suggests that such an explanation is unlikely to explain our key results. However, the widespread translocations of water frogs is well recorded, and the impact that these invasions may have on local haplotypes is an increasing cause of concern for this genus (Dubey *et al* 2014, Dufresnes *et al* 2017, 2020).

Finally, it is possible that pool frogs are relatively genetically homogenous throughout their range, and do not form distinct genetic clades, as might be expected if colonisation of most of Europe was relatively rapid. Future phylogenies incorporating additional, more variable, genetic markers will be able to test this possibility. Indeed, a recent attempt to resolve *Pelophylax* relationships using both mitochondrial and nuclear DNA reveals *very* recent divergence of *P. lessonae* lineages (~300kya for earliest divergences; Dufresnes *et al* 2024) and as my results suggest that divergence across most of Europe happened much more recently than the earliest divergences, this is consistent with rapid dispersal across the continent. The inclusion of more diverse markers may be particularly important to uncover fine-scale differences involving northern populations as they have particularly low levels of genetic diversity in comparison to their mainland counterparts (Sjögren 1991). Indeed, previous studies based on microsatellite markers find that northern populations contain a subset of the alleles that are present in mainland populations (Zeisset and Beebee 2001), likely due to them being present in small populations at the range edge (Sjögren 1991, Cahill and Levinton 2015). Regardless of whether or not they form a single distinct genetic unit, the northern pool frog can still be considered to be important from a conservation perspective as they are divergent in terms of their appearance, vocalisations and behaviour (Wycherly *et al* 2002, Haugen 2020, Snell *et al* 1994), as well as their lack of recent hybridisation with other *Pelophylax* species (Zeisset and Hoogerstege 2018, Zeisset and Beebee 2001, Sjögren 1991).

Furthermore, the tree presented in this thesis could be made more informative in relation to the history of the species by creating a time-calibrated phylogeny, using more *Pelophylax* species to root the tree and provide additional calibration points for divergence time estimation. This may provide further insight into when pool frogs colonised northern Europe and even when hybridisation began in the genus, as northern populations likely became isolated before this happened. However, due to the time constraints of this project, these further steps could not be included in this study.

4.2 Climatic niches of northern and mainland pool frogs

The BRT species distribution models suggested that for pool frogs across their full range, annual mean temperatures of around 5-10°C, and high seasonal changes in precipitation, appear to positively influence occupation by the species, with altitude playing little part in affecting occurrence. Despite this they appear to occupy a wide range of climatic conditions and are widely distributed throughout Europe, attesting to the diversity within this species and *Pelophylax* hybrid complex in general. Complex genome exchanges between *Pelophylax* species and hybrids may maintain genetic diversity and drive a diversity in climate and niche preferences, much like in the *Ambystoma* species complex does in North America (Uzzel 1964). This may help to sustain the *Pelophylax* species complex in the face of anthropogenic climate change. Nevertheless, hybridisation has been proposed to threaten some populations of pool frogs with genetic exclusion (Christiansen 2009, Holsbeek and Joris 2010, Dubey 2014), emphasising the importance of conserving non-hybridising pool frog populations such as those in northern latitudes and Corsica.

Using data from only northern populations, I found altitude to be the strongest predictor of frog presence, with populations being limited to low elevation habitats with relatively low precipitation. This means that northern populations of pool frogs in their native range are limited to low lying coastal habitat, with relatively little snowfall, and where they can hibernate through winter temperatures that are often below freezing provided the breeding season is relatively warm and with low precipitation. Despite finding some differences in the environmental conditions associated with their distribution between mainland pool frogs and non-hybrid northern populations, I also found substantial niche overlap, with northern populations occupying a similar but narrower niche. This close similarity is to be expected as this clade have been shown to genetically vary little from mainland pool frogs, but their populations contain far less diversity (Sjögren 1991). The lack of genomic exchange with other *Pelophylax*, as well as the isolated, edge-range nature of northern populations may have resulted in a narrower niche which fits within the larger niche of the species in general.

The species distribution models found suitable habitat for pool frogs on both sides of the North Sea, but with northern pool frogs on one side (British and Scandinavia), and hybrid mainland pool frogs on the other (France, Netherlands, Denmark etc). Archaeological and historical evidence suggests that pool frogs occurred in much of East Anglia and Lincolnshire prior to anthropogenic

reintroductions (Gleed Owen 2000, Raye 2017). The detailed maps of suitable environmental conditions across the UK may reveal other areas where native pool frogs may have once occurred, such as the long-urbanised Thames estuary, where invasive water frogs now thrive (Langton *et al* 2011), much of the south coast, and the Mersey and Dee estuaries. These locations host the highest exotherm vertebrate diversity in the British Isles, hosting edge-range populations of sand lizard (*Lacerta agilis*), smooth snake (*Coronella austriaca*) and natterjack toad (*Epidalea calamita*) (Arnold 1995), and may provide suitable future potential reintroduction sites for pool frogs in the UK. Future studies of archaeological samples would be useful in revealing whether native pool frogs occupied these areas in the past, thus informing future reintroduction projects.

The Species Distribution Models also faced some limitations, the most fundamental of which is reliable occurrence data. First, *Pelophylax* species and hybrids are inherently tricky to identify morphologically. Therefore, the occurrence data across much of mainland Europe may also include other *Pelophylax spp* in addition to pool frogs. However, an aim of this study was to find out how the single-species northern populations may differ from their hybrid conspecifics, so the presence of hybrids may not have a large impact on the conclusions. Nevertheless, this issue highlights the need for more reliable techniques in identifying these cryptic species. This brings us onto the second limitation with the occurrence data; defining northern populations. As stated in the methods (Section 2.2), I took a generous approach to assigning occurrence data to the northern populations, based on Zeisset and Hoogersteger (2018), for the purpose of maximising sample size. However, this could include introduced, non-northern *Pelophylax* populations as the origin of many populations has not been tested. This raises the need for a better understanding of where non-hybridising populations exist, as the predicted suitable climates for northern pool frogs span across much of the North Sea and Baltic coastline.

Despite the predicted available climates, frog distribution is also limited by other environmental factors such as suitable water bodies or competition with other *Pelophylax spp*. My testing for model fit revealed that the models performed around 0.6-0.7 AUC, meaning the fit was less than halfway between random guessing and a perfect fit. This suggests that other factors not included in the models may play a large part in influencing occurrence for the species. For example, competition, particularly from *P.kl.esculenta* and *Pridibundus*, likely has a significant role (Pille 2024), and details of the habitat (e.g. the presence of suitable watercourses) is likely to be very important in explaining the distribution of the species within its broad climatic niche. Future distribution models incorporating finer scale habitat features will be able to provide better resolution and further insight into potential reintroduction sites and localities to direct conservation resources towards. This could be done by using satellite image derived habitat data as a predictor such the Living England habitat map (<https://naturalengland-defra.opendata.arcgis.com/maps>), or better still by mapping ponds and water ways with finer scale habitat data, these features are essential to water frogs and so habitat and microclimate data for these would likely be much more influential to their occurrence than the broad scale climatic variables used in this study.

4.3. Summary and conclusions

Pool frogs showed relatively little diversity and structure across the 50 CytB sequences tested in our phylogeny making a colonisation path difficult to infer, although sequences from Denmark and Italy appear to be ancestral to the rest. This may be due to the mtDNA being conserved across hybrid systems within the less diverse *P. lessonae* genome.

Pool frogs exhibit a broad climate niche, with occurrence spanning a wide range of temperatures, precipitations, and altitudes. This means that for the species as a whole, suitable climates are widespread across Europe. However, northern pool frogs seemingly possess a narrower niche that fills a fraction of the larger one. This may be due to these populations having limited diversity brought on by their isolation at the end of their range. In these northern, edge-range habitats pool frogs are strongly limited to low lying coastal areas, which experience low precipitation and so will be free of snow for extended periods of the warmer months.

The species distribution models predict suitable climate conditions for northern pool frogs across much of eastern and south eastern England, with the most suitable areas correlating with historic records of the species and places where pool frogs breed today. This infers that the native range of pool frogs in Britain likely covered East Anglia, southern Lincolnshire and the Thames estuary, and so conservation efforts should focus on restoring the species and its habitat to these areas, whilst also considering the populations of non-native *Pelophylax spp* and mainland European introduced pool frog populations, which are present in some of these areas and could therefore interbreed with any reintroduced northern populations.

The future of the pool frog in Britain holds great importance for the conservation of the species in general. With reported declines in the already small populations in Scandinavia, and threats such as disease and invasive species it becomes vital to restore pool frogs to their native range within England (Wilkinson and Buckley 2012). With this in mind, a dynamic response to their conservation is important, and I hope that the information this study provides about the climatic preferences of northern pool frogs, can be useful when selecting new areas to focus conservation efforts. Future work would be well placed to investigate habitat in finer scale, such as identifying native communities that characterise ecosystems that can best support pool frogs. Climate change is also an increasing concern and may be shifting the geographic distribution of suitable climates for pool frogs. Extending species distribution models to incorporate predicted future climates could further inform a long-term plan for the recovery of the species in the UK and across other northern populations.

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1. Appendices:

Appendix A: Sequence Data downloaded from NCBI GenBank for phylogenetic analysis, with references ascension number, sequences length and species.

GenBank Ascension number	Length (bp)	Species
OQ130160.1	1143	<i>P.cf. lessonae</i>
MT737327.1	878	<i>P.cf. lessonae</i>
MT737328.1	873	<i>P.cf. lessonae</i>
MT737329.1	873	<i>P.cf. lessonae</i>
MT737330.1	880	<i>P.cf. lessonae</i>
MT737331.1	789	<i>P.cf. lessonae</i>
MT737332.1	798	<i>P.cf. lessonae</i>
MT737333.1	707	<i>P.cf. lessonae</i>
MT737335.1	840	<i>P.cf. lessonae</i>
MT737336.1	870	<i>P.cf. lessonae</i>
MT737338.1	922	<i>P.cf. lessonae</i>
MT737339.1	833	<i>P.cf. lessonae</i>
MT737341.1	778	<i>P.cf. lessonae</i>
MT737342.1	873	<i>P.cf. lessonae</i>
MT737343.1	873	<i>P.cf. lessonae</i>
MT737344.1	873	<i>P.cf. lessonae</i>
MT737345.1	879	<i>P.cf. lessonae</i>
MT737346.1	866	<i>P.cf. lessonae</i>
MT737348.1	870	<i>P.cf. lessonae</i>
MT737350.1	877	<i>P.cf. lessonae</i>
MT737352.1	873	<i>P.cf. lessonae</i>
MT737351.1	872	<i>P.cf. lessonae</i>
MT737356.1	978	<i>P.cf. lessonae</i>
MT737357.1	984	<i>P.cf. lessonae</i>
MT737358.1	924	<i>P.cf. lessonae</i>
MT737359.1	914	<i>P.cf. lessonae</i>
LC599369.1	492	<i>P.cf. lessonae</i>
LC599370.1	492	<i>P.cf. lessonae</i>
LC599371.1	492	<i>P.cf. lessonae</i>
LC599372.1	492	<i>P.cf. lessonae</i>
LC599373.1	492	<i>P.cf. lessonae</i>
AB980791.1	1098	<i>P.cf. lessonae</i>
MG214959.1	552	<i>P.cf. lessonae</i>
MG214960.1	552	<i>P.cf. lessonae</i>
MG214961.1	552	<i>P.cf. lessonae</i>
MH410381.1	563	<i>P.cf. lessonae</i>
MH410415.1	563	<i>P.cf. lessonae</i>

MH410416.1	563	<i>P.cf. lessonae</i>
MH410420.1	563	<i>P.cf. lessonae</i>
JQ282930.1	521	<i>P.cf. lessonae</i>
JQ282971.1	521	<i>P.cf. lessonae</i>
KY129866.1	675	<i>P.cf. lessonae</i>
KY129867.1	675	<i>P.cf. lessonae</i>
KY129868.1	675	<i>P.cf. lessonae</i>
KY129869.1	675	<i>P.cf. lessonae</i>
KY129870.1	675	<i>P.cf. lessonae</i>
KY703611.1	797	<i>P.cf. lessonae</i>
AY043051.1	275	<i>P.cf. lessonae</i>
AF084047.1	293	<i>P.cf. lessonae</i>
AB980716.1	1098	<i>P.cf. lessonae</i>
MF667559.1	968	<i>P. perezi</i>
MF667560.1	974	<i>P. perezi</i>
MF667561.1	974	<i>P. perezi</i>
MF667562.1	974	<i>P. perezi</i>
MF667563.1	974	<i>P. perezi</i>
MF667564.1	974	<i>P. perezi</i>
MF667565.1	974	<i>P. perezi</i>
MF667566.1	974	<i>P. perezi</i>
MF667567.1	974	<i>P. perezi</i>
MF667568.1	974	<i>P. perezi</i>
MF667569.1	974	<i>P. perezi</i>
MF667570.1	974	<i>P. perezi</i>
MF667571.1	971	<i>P. perezi</i>
MF667572.1	974	<i>P. perezi</i>
MF667573.1	974	<i>P. perezi</i>
MF667574.1	974	<i>P. perezi</i>
MF667575.1	974	<i>P. perezi</i>
MF667576.1	974	<i>P. perezi</i>
AY043052.1	275	<i>P. perezi</i>
DQ902145.1	984	<i>P. perezi</i>

Appendix B: Phylogeny Reconstruction Model Performance statistics. GTR - General Time Reversible model, HKY - Hasegawa Kishino Yano model, HKY+G - HKY model with gamma-distributed rates across sites, HKY+G+I - HKY model with both gamma-distributed rates and invariant sites, TN93+G - Tamura-Nei model with gamma-distributed rates, TN93+G+I - Tamura-Nei model with gamma-distributed rates and invariant sites, HKY+I - HKY model with invariant sites, GTR+G - GTR model with gamma-distributed rates, GTR+G+I - GTR model with gamma-distributed rates and invariant sites, TN93+I - Tamura-Nei model with invariant sites, GTR+I - GTR model with invariant sites, HKY - Hasegawa Kishino Yano model, TN93 - Tamura-Nei model, K2+G - Kimura 2-parameter model with gamma-distributed rates, T92+G - Tamura 1992 model with gamma-distributed rates, K2+G+I - Kimura 2-parameter model with gamma-distributed rates and invariant sites, T92+G+I - Tamura 1992 model with gamma-distributed rates and invariant sites, GTR - General Time Reversible model, K2+I - Kimura 2-parameter model with invariant sites, T92+I - Tamura 1992 model with invariant sites, K2 - Kimura 2-parameter model, T92 - Tamura 1992 model, JC+G - Jukes-Cantor model with gamma-distributed rates, JC+G+I - Jukes-Cantor model with gamma-distributed rates and invariant sites, JC+I - Jukes-Cantor model with invariant sites, JC - Jukes-Cantor model.

Model	#Param	BIC	AICc	lnL	Invariant	Gamma	R
HKY+G	142	7275.327421	6007.696102	-2861.484151	n/a	0.227391599	3.60418674
HKY+G+I	143	7284.302333	6007.749172	-2860.505554	0.445714898	0.628394688	3.77704229
TN93+G	143	7285.908415	6009.355255	-2861.308595	n/a	0.22922259	3.64210971
TN93+G+I	144	7294.939138	6009.464207	-2860.357903	0.441157222	0.624827636	3.79692239
HKY+I	142	7312.645289	6045.013971	-2880.143085	0.4069869	n/a	2.89200586
GTR+G	146	7313.158866	6009.84061	-2858.535661	n/a	0.23338878	3.60481606
GTR+G+I	147	7322.442304	6010.202493	-2857.711326	0.424719584	0.626899873	3.69008818
TN93+I	143	7322.690464	6046.137303	-2879.699619	0.4069869	n/a	2.91378128
GTR+I	146	7349.179654	6045.861398	-2876.546055	0.4069869	n/a	2.91573456
HKY	141	7350.721955	6092.012552	-2904.647472	n/a	n/a	2.66323546
TN93	142	7360.840574	6093.209256	-2904.240728	n/a	n/a	2.66735467
K2+G	139	7365.162512	6124.297153	-2922.799856	n/a	0.227620384	3.60049607
T92+G	140	7371.925815	6122.138398	-2920.715455	n/a	0.228453051	3.59780751
K2+G+I	140	7374.786975	6124.999558	-2922.146035	0.451521556	0.672869344	3.71651087
T92+G+I	141	7381.856029	6123.146626	-2920.214509	0.456272915	0.672330048	3.77051859
GTR	145	7386.866493	6092.469863	-2900.855527	n/a	n/a	2.67134186
K2+I	139	7402.061737	6161.196379	-2941.249469	0.4069869	n/a	2.88056439
T92+I	140	7408.795642	6159.008225	-2939.150368	0.4069869	n/a	2.87959047
K2	138	7439.155837	6207.21261	-2965.262572	n/a	n/a	2.65793198
T92	139	7445.92348	6205.058122	-2963.18034	n/a	n/a	2.65628408
JC+G	138	7523.13867	6291.195443	-3007.253989	n/a	0.261762515	0.5
JC+G+I	139	7543.499648	6302.63429	-3011.968424	0.461097974	0.491405793	0.5
JC+I	138	7558.595973	6326.652745	-3024.98264	0.4069869	n/a	0.5
JC	137	7588.132569	6365.111544	-3045.216991	n/a	n/a	0.5