

1 **An integrated framework to identify wildlife populations under threat from**
2 **climate change**

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31 **Running title:** A framework to evaluate climate change threat

32 **Abstract**

33 Climate change is a major threat to global biodiversity that will produce a range of new
34 selection pressures. Understanding species responses to climate change requires an
35 interdisciplinary perspective, combining ecological, molecular and environmental approaches.
36 We propose an applied integrated framework to identify populations under threat from
37 climate change based on their extent of exposure, inherent sensitivity due to adaptive and
38 neutral genetic variation and range shift potential. We consider intraspecific vulnerability and
39 population-level responses, an important but often neglected conservation research priority.
40 We demonstrate how this framework can be applied to vertebrates with limited dispersal
41 abilities using empirical data for the bat *Plecotus austriacus*. We use ecological niche
42 modelling and environmental dissimilarity analysis to locate areas at high risk of exposure to
43 future changes. Combining outlier tests with genotype-environment association analysis we
44 identify potential climate-adaptive SNPs in our genomic dataset and differences in the
45 frequency of adaptive and neutral variation between populations. We assess landscape
46 connectivity and show that changing environmental suitability may limit the future movement
47 of individuals, thus affecting both the ability of populations to shift their distribution to
48 climatically suitable areas and the probability of evolutionary rescue through the spread of
49 adaptive genetic variation among populations. Therefore a better understanding of movement
50 ecology and landscape connectivity is needed for predicting population persistence under
51 climate change. Our study highlights the importance of incorporating genomic data to
52 determine sensitivity, adaptive potential and range shift potential, instead of relying solely on
53 exposure to guide species vulnerability assessments and conservation planning.

54 **Introduction**

55 Climate change is a major threat to global biodiversity (IPCC, 2013). Increased periods of
56 drought, thermal stress and extreme climatic events are likely to produce a range of new
57 selection pressures (Hoffmann & Sgrò 2011). The ability of populations to respond to these
58 changes depends on the rate and magnitude of climate change and individual adaptive
59 capacity based on physiological sensitivity to change, phenotypic plasticity, genetic diversity
60 and dispersal ability (Dawson *et al.* 2011). Many species are already affected by climate
61 change, and as a result have displayed a variety of responses, including shifting their ranges
62 and changes to phenotypes, genotypes, growth, phenology and ecological relationships
63 (Peñuelas *et al.* 2013). Hence understanding how biodiversity responds to climate change
64 requires an interdisciplinary perspective, combining ecological, molecular and environmental
65 approaches, and an integrated assessment of exposure to changing climatic conditions,
66 adaptive potential and movement ability. Yet while exposure is commonly used to assess
67 species vulnerability to climate change, the other aspects of vulnerability, sensitivity and
68 adaptive potential, have been largely neglected, thus precluding accurate estimations of
69 species-specific vulnerability (Butt *et al.* 2016).

70 Ecological niche models (ENMs), also known as species distribution models, offer an
71 effective tool for forecasting how climate change may alter future species distributions and
72 patterns of diversity (Elith *et al.* 2010). ENMs have been used extensively to identify species
73 vulnerable to future changes (Pacifiçi *et al.* 2015) and predict global patterns of extinction
74 risk (Urban, 2015). Their popularity is attributed to the availability of fine-scale climate
75 change scenarios, the relative simplicity of the modelling procedures and the lack of detailed
76 physiological and life history data necessary for parameterising more complex mechanistic or

77 demographic models (Guisan & Thuiller 2005; Thuiller *et al.* 2013). However, predictive
78 modelling studies have been criticised for being over-simplistic because they rarely address
79 evolutionary processes (Thuiller *et al.* 2013) or integrate genetic data to support and validate
80 predictions (Gotelli & Stanton-Geddes 2015).

81 The study of local adaptations to current climatic gradients can contribute to understanding
82 the ability of populations to persist or adapt to rapid environmental change (Fournier-Level *et*
83 *al.* 2011). Intraspecific variation in climatic tolerance will result in different responses to
84 climate change below the species level, and therefore geographic areas that are most sensitive
85 can be identified through mapping spatial patterns of local adaptations (Fitzpatrick & Keller
86 2015). Recent technological advances and theoretical developments enable investigation of
87 the genetic basis of adaptations and mechanisms of adaptive responses in wild populations
88 (Orsini *et al.* 2013; Andrews *et al.* 2016). Studies researching patterns of genome variation
89 demonstrated how adaptations to climatic conditions can shape the spatial distribution of
90 variation in plants (*Arabidopsis thaliana*, Fournier-Level *et al.* 2011) and humans (Hancock *et*
91 *al.* 2011). However, most research to date has focused mainly on model organisms and on
92 genes thought to be involved in adaptations to current environmental conditions, rather than
93 predicting responses to future conditions (Manel & Holderegger 2013).

94 While some populations can persist through available genetic variation or their adaptive
95 capacity, the persistence of many individuals depends on their ability to track suitable
96 conditions in space through dispersal or by shifting to different habitats (Bellard *et al.* 2012).
97 Understanding dispersal is important for predicting species responses to environmental
98 change because it determines both the rate of distributional shifts and the rate of evolutionary
99 adaptation to changing conditions through the spread of adaptive alleles among populations

100 (Travis *et al.* 2013). Landscape genetics, the study of the effects of environmental
101 heterogeneity on the spatial distribution of genetic variation (Manel *et al.* 2003), can help
102 identify barriers to dispersal that are likely to limit species ability to respond to climate
103 change through tracking changes to their environmental niche (Scoble & Lowe 2010). A
104 further, yet unexplored, application is to infer the effect of landscape connectivity on the
105 probability of evolutionary adaptation through the spatial spread of adaptive variation
106 between populations. Despite its potential as a predictive tool, thus far landscape genetics has
107 been primarily applied in a descriptive manner (Manel & Holderegger 2013).

108 We propose an applied framework that integrates ecological, molecular and environmental
109 approaches to identify populations under threat from global climate change. Unlike previous
110 climate change vulnerability assessments (e.g. Pearson *et al.* 2014; Pacifici *et al.* 2015), we
111 consider the intraspecific level because populations will go extinct long before species, and it
112 is populations, not species, that are the focus of conservation management. Our framework
113 aims to address the lack of emphasis on sensitivity and adaptive capacity in vulnerability
114 assessments used to inform conservation planning under climate change (Butt *et al.* 2016).

115 We assess exposure to changing climatic conditions using predictive ENMs and spatial
116 environmental data, sensitivity to climate change using genomic data to identify climate-
117 driven genetic adaptations, and range shift potential using a predictive landscape genetics
118 approach (Fig. 1). This framework is aimed at organisms that are unlikely to genetically adapt
119 fast enough through the spread of novel mutations in the population to keep pace with future
120 changes due to their relatively long lifespans, long generation times and small population
121 sizes (i.e. most vertebrates; Hoffmann & Sgrò 2011). Therefore instead of emphasising
122 general adaptive capacity, like previous conceptual frameworks have done (e.g. Dawson *et al.*

123 2011), we focus on the ability to track future climatic suitability (range shift potential) and
124 evolutionary adaptation through the spread of adaptive genetic variation among populations.

125 We apply our integrated framework to the grey long-eared bat, *Plecotus austriacus*, a
126 European bat species with relatively limited dispersal ability that is of conservation concern at
127 the northern parts of its range (Van der Meij *et al.* 2015). We selected this species because its
128 geographic distribution is limited by climate and its current patterns of genetic variation were
129 shaped by past climatic changes (Razgour *et al.* 2013). Bats possess a number of traits that
130 make them vulnerable to climate change, including low reproductive output, ecological
131 specialisation and high trophic positions (Jones & Rebelo 2013). High surface-to-volume
132 ratios due to large membranous, non-insulated wings, means that evaporative water loss is
133 higher in bats than in other small mammals (Webb *et al.* 1995). As a result bats may require
134 specific physiological adaptations to cope with increased temperatures and aridity (Muñoz-
135 Garcia *et al.* 2016). We aim to identify *P. austriacus* populations vulnerable to future climate
136 change based on their extent of exposure to changing climatic conditions, sensitivity due to
137 adaptive and neutral genetic variation and range shift potential. We use this case study to
138 demonstrate how our integrated framework can inform conservation management under
139 global environmental change.

140 **Materials and Methods**

141 **Sampling design**

142 We collected non-lethal tissue samples (wing biopsies) from *Plecotus austriacus* bats,
143 sampled between 2009 and 2013 from across the Iberian Peninsula (Iberia) and the south of
144 England. These areas represent the species' southern and northern range limits, as well as the

145 centre and margin of the species' ecological niche, respectively. We included 10 populations
146 (eight from Iberia, two from England) that had at least eight individuals with sufficiently high
147 DNA quantity and quality (N=94). These populations represent different geographical areas
148 and combinations of climatic conditions (Fig. 2; Table 1). All populations were located more
149 than 90 km apart, exceeding the maximum recorded dispersal distance in this species (62 km;
150 Riede 2001).

151 **Assessing exposure: environmental changes and ecological niche modelling**

152 ENMs were generated using the maximum entropy modelling approach Maxent v3.3.3
153 (Phillips *et al.* 2006) to predict changes to the distribution of suitable conditions for *P.*
154 *austriacus* under future climate change projections. Model extent was set as the whole of
155 Europe to account for the full range of environmental conditions experienced by the species.
156 Model resolution was set at ~1km to match the resolution of the climatic data used in the
157 sensitivity analysis. ENMs were built using 142 genetically confirmed non-clustered location
158 records (Razgour *et al.* 2013) and included six climatic variables with future projections for
159 2070, a static topographic variable, slope, and a land cover variable with no fine-scale future
160 projections (Table S1). We included land cover because such non-climatic variables can
161 greatly improve ENM predictive performance, even in the absence of future projections
162 (Stanton *et al.* 2012). Future projections were carried out using the HadCM3_ES General
163 Circulation Model (www.worldclim.org) and the IPCC5 +8.5 W/m² Representative
164 Concentration Pathways scenario (IPCC 2013), representing the 'worst case' scenario,
165 whereby human consumption of fossil fuels is expected to remain the same as at present
166 (Appendix 1 for further details on variable selection and model parameterisation).

167 The predictive power of the ENMs was evaluated from ten cross-validations, using the Area
168 Under the Receiver Operator Curve (AUC) criteria. Climatic suitability was determined based
169 on predicted relative probability of occurrence and was averaged across the four cells adjacent
170 to the population location to cover the colony home range (Razgour *et al.* 2011). To calculate
171 changes in range suitability within Iberia, continuous occurrence probability model outputs
172 were reclassified into binary maps using the thresholding method that maximises the sum of
173 sensitivity and specificity (as recommended by Liu *et al.* 2013).

174 Maximum temperatures and summer rainfall (Bio5 and Bio18, downloaded from WorldClim,
175 www.worldclim.org) were used to test for environmental dissimilarity between current and
176 future (2070) conditions. These variables represent climatic conditions that are predicted to
177 change under future projections for Iberia (Diffenbaugh & Field 2013) and are likely to affect
178 bats. Increased aridity and prolonged droughts around the Mediterranean are predicted to
179 affect insect prey availability during the summer (Frampton *et al.* 2000), and thus decrease
180 reproductive success in bats (Adams 2010; Amorim *et al.* 2015). In addition, bat survival in
181 warmer and more arid conditions requires physiological adaptations to reduce evaporative
182 water loss (Muñoz-García *et al.*, 2016).

183 **Assessing sensitivity: genomic data analysis**

184 We generated a genomic dataset containing thousands of anonymous genetic loci from across
185 the *P. austriacus* genome using the reduced-representation genome sequencing method
186 double digest Restriction-site Associated DNA Sequencing, ddRADseq (Miller *et al.* 2007;
187 Peterson *et al.* 2012; library construction and sequencing protocols outlined in Appendix 1).
188 Bioinformatics of the high throughput sequencing data was carried out using the STACKS
189 pipeline (Catchen *et al.* 2013; details in Appendix 1). To improve robustness of the dataset

190 only RAD loci that contained fewer than three SNPs, and were genotyped in at least 70% of
191 the samples (67 individuals) were considered for analysis. The SNP dataset was processed in
192 Plink v1.9 (Purcell *et al.* 2007) to remove individuals that had more than 50% missing data
193 and loci with more than 30% missing data and minor allele frequencies below 0.03 (alleles
194 present in less than three individuals). We also removed close relatives (based on identity-by-
195 state distances, PI HAT >0.5) and loci that were out of Hardy-Weinberg equilibrium (P
196 <0.01) in more than two populations. Population-level analyses were carried out on
197 populations containing a minimum of seven individuals to ensure an adequate representation
198 of allele frequencies (Willing *et al.* 2012).

199 Genetic population structure was determined using individual-based Bayesian assignment
200 tests, implemented in the programme STRUCTURE v2.3.4 (Pritchard *et al.* 2000) (Appendix
201 1 for STRUCTURE running procedures). The significance of genetic differences between
202 populations and geographic regions (England versus Iberia) was determined based on a
203 multilocus Analysis of Molecular Variance (AMOVA) implemented in the R package
204 GStudio (Dyer 2009).

205 *Identifying a signature of climate-driven adaptations*

206 To identify a signature of climate-driven local adaptations we combined population genomics
207 and ecological approaches. Outlier tests, as implemented in the programmes Bayescan (Foll &
208 Gaggiotti 2008) and LOSITAN (FDist, Antao *et al.* 2008) were used to identify SNPs
209 potentially under directional selection, or linked with genes under selection, based on higher
210 levels of genetic differentiation among populations relative to expected neutral distributions
211 (Appendix 1 for test parameters). Allele frequencies of SNPs identified as outliers were

212 correlated against environmental variables (maximum temperature and summer rainfall) using
213 logistic regressions (glm function in R), as described by Schoville *et al.* (2013).

214 We carried out a genotype-environment association analysis to test for associations between
215 allele frequencies and local environmental variables (maximum temperature and summer
216 rainfall). We used the latent factor mixed model (LFMM) approach, implemented in the R
217 package LEA (Frichot & François 2015). We corrected for population structure through
218 including the number of populations (K) identified by STRUCTURE assignment tests as
219 latent factors in the models. We performed five LFMM repetition runs with 1,000,000
220 iterations and 500,000 iterations for burn-in. Z-scores of multiple runs were combined using
221 the median value and p-values were adjusted for expected FDR of 0.05 (following the
222 procedures in Frichot & François 2015; Appendix 2 for LFMM R script). SNPs that were
223 found to be both under directional selection based on outlier tests and statistically associated
224 with climatic variables based on the genotype-environment association analysis were
225 classified as potentially associated with climate-adaptive genetic variation, i.e. with
226 adaptations to local climatic conditions. However it is important to note that these SNPs may
227 represent genomic regions linked to genes under selection rather than specific climate-
228 adaptive genes.

229 Genotype-environment associations between SNPs and climatic variables were investigated at
230 two scales, across the whole study area (England and Iberia) and within Iberia, to account for
231 clines in allele frequencies at neutral loci due to genetic drift and allele surfing during
232 population expansion (Excoffier & Ray 2008). The Iberian Peninsula acted as the main glacial
233 refugium for *P. austriacus*, where a stable population was maintained across glacial cycles
234 (Razgour *et al.* 2013). Hence SNPs identified as potentially under selection within this area

235 likely reflect true climate-driven adaptations rather than artefacts of neutral processes that
236 occurred during post-glacial range expansion.

237 *Patterns of neutral genetic variation*

238 Neutral genetic diversity was estimated based on levels of heterozygosity in the population
239 after excluding SNPs identified as outliers (under selection) by Bayescan. We used the --het
240 function in Plink to compare observed and expected individual levels of homozygosity.
241 Heterozygosity was calculated as $1 - (mean\ population\ F)$, F being the coefficient estimation
242 of observed (Obs) versus expected (Exp) homozygosity (Hom):

$$243 F = (Obs_Hom - Exp_Hom) / (Total - Exp_Hom).$$

244 **Assessing range shift potential: landscape genetics analysis**

245 Genetic distances between pairs of populations were estimated separately for the neutral SNP
246 dataset and for SNPs identified as a potentially under climate-driven selection, using the F_{st}
247 measure of genetic differentiation in the R package *diveRsity* (Keenan *et al.* 2013).
248 Geographic (Euclidean) distances between populations were calculated in ArcGIS v10
249 (ESRI). The analysis included landscape variables and resistance costs that were previously
250 shown to affect functional connectivity in *P. austriacus* (Razgour *et al.* 2014; Razgour 2015):
251 habitat suitability measured through ENMs, forest cover variables, altitude and slope. We did
252 not include landscape variables that were highly correlated with other variables or geographic
253 distance ($R^2 > 0.70$) because they can lead to the identification of spurious inferences
254 (Cushman *et al.* 2013).

255 Landscape variables were converted to resistance cost surfaces in ArcGIS and were assigned
256 resistance costs ranging from one (no resistance to movement) to 100 (strong barrier to

257 movement). The sea was assigned a resistance cost of 200 to reflect the lower likelihood of
258 bats crossing large expanses of water than land because previous studies have found limited
259 gene flow across seas in this species (Razgour *et al.* 2014). We tested the effect of decreasing
260 the resistance costs of crossing the sea to 120. We tested how changing the resistance costs of
261 the different landscape variables and converting continuous into categorical variables affected
262 the strength of the model associations with genetic differentiation (Appendix 1 for generating
263 resistance cost surfaces).

264 Circuitscape v4.0.5 (McRae 2006) was used to calculate resistance distance matrices between
265 populations and estimate potential movement pathways across the landscape based on the
266 cumulative cost of movement due to landscape resistance. We used the nine populations as
267 our focal nodes and selected the ‘pairwise’ modelling mode (iterating across all population
268 pairs in focal node file). Movement pathways (cumulative current maps) were generated
269 based on present and future (2070) conditions to assess the future movement potential of
270 individuals and adaptive genetic variation among populations.

271 We used Multiple Regressions on Distance Matrices (MRDM in the R package *ecodist*;
272 Goslee & Urban 2007) with 10,000 permutations to test for the effect of landscape variables
273 on genetic differentiation (as a surrogate for gene flow and individual movement) between
274 population pairs. We ran MRDM between F_{st} and all landscape variables and their different
275 resistance costs to select the resistance cost combinations that showed the strongest
276 correlations. Following Dyer *et al.* (2010), we accounted for the effect of geographic distance
277 using a stepwise approach. We first ran MRDM between F_{st} and geographic distance, and
278 then used the residuals from the regression as the response variable in subsequent MRDM
279 models to test for associations with landscape variables. The best-fit model was selected

280 based on highest R^2 values and significant P values for all variables ($P < 0.05$). MRDM was
281 also used to test whether genetic differentiation (F_{st}) between populations in climate-adaptive
282 SNPs was a function of environmental dissimilarity (differences in maximum temperature and
283 summer rainfall) between locations (isolation by environment).

284 **Identifying level of risk**

285 We developed a quantitative approach to identify the level of risk to populations from future
286 climate change based on our three framework components, exposure, sensitivity and range
287 shift potential. Assigned levels of risk aim to guide conservation prioritisation and inform
288 management decisions through highlighting which aspects should be the focus of
289 conservation action.

290 Exposure was ranked from low (1) to high (4) based on changes in climatic suitability as
291 predicted by the ENMs (reduction in relative probability of occurrence and changes from
292 suitable to unsuitable conditions) and the extent of environmental dissimilarity between
293 present and future conditions (Table 2).

294 Sensitivity was determined based on the frequency of alleles in SNPs identified as potentially
295 associated with warmer and drier climatic conditions (adaptive sensitivity), as well as overall
296 levels of neutral genetic diversity (neutral sensitivity). Levels of adaptive sensitivity were
297 determined based on the frequency of potential climate-adaptive alleles in the population,
298 looking at both overall mean frequencies across all loci (high [++ or +] < 0.50 ; medium [0] –
299 low [-] > 0.50), and number of adaptive alleles present at particularly low frequencies (< 0.25)
300 in the population (Table 3). Levels of neutral sensitivity were assessed based on the potential
301 contribution of neutral genetic diversity to future adaptive potential (- low sensitivity due to

302 high levels of neutral genetic diversity; 0 medium sensitivity; + high sensitivity due to
303 relatively low levels of genetic diversity). The two measures were combined together to give
304 a single measure of overall sensitivity.

305 Range shift potential was determined according to the degree of connectivity to other
306 populations under future conditions or to areas predicted to be climatically suitable, as
307 estimated based on Circuitscape movement density (cumulative current) maps. Populations
308 were deemed to have low range shift potential if they were predicted to become isolated (low
309 connectivity) under future conditions, or only connected to adjacent populations and
310 climatically unsuitable areas.

311 The three framework components were combined together to determine the level of risk to
312 Iberian populations of *P. austriacus*, ranging from low to high risk. When combining the
313 three components we placed higher weights on exposure to future changes in climatic
314 conditions (Table 4).

315 **Results**

316 **Exposure to climate change**

317 ENMs had high discrimination and predictive abilities ($AUC_{\text{train}}=0.892$, $ACU_{\text{test}}=0.821$). The
318 main variables affecting habitat suitability for *P. austriacus* were winter and summer
319 temperatures, summer rainfall and land cover (Fig. S1). Future models predicted 66.3%
320 reduction in the suitable range of *P. austriacus* within Iberia. Under present conditions, 54.8%
321 of Iberia was predicted to be climatically suitable, but only 18.5% was predicted to remain
322 suitable by 2070. All populations were found within climatically suitable areas under present
323 conditions. Under future conditions, five Iberian populations were predicted to occur either in

324 climatically unsuitable areas (Albacete, Valencia and Girona) or in small isolated fragments
325 of suitable habitats (Granada and Valladolid). Habitat suitability was predicted to increase
326 slightly in England and around the north Iberian coast (Fig. 3).

327 Analysis of environmental dissimilarity between current and future conditions predicted that
328 the central and eastern populations (Valladolid, Albacete and Valencia) will experience the
329 greatest increase in maximum temperatures ($>7^{\circ}\text{C}$) and the greatest proportional reduction in
330 summer rainfall (48-55%; Table 5). Based on ENM predicted changes in climatic suitability
331 and the extent of environmental dissimilarity between present and future conditions, two
332 populations were assigned high exposure values (Albacete and Valencia), two medium-high
333 (Girona and Valladolid), one medium-low (Granada) and two low (Lisboa and Bizkaia; Table
334 6).

335 **Assessing sensitivity to climate change**

336 From the high throughput sequencing we identified 39,825,843 de-multiplexed, paired-end
337 reads, from which Stacks resolved 11,116 RAD tags that were present in at least 70% ($n=67$)
338 of all individuals screened and contained a maximum of 3 SNPs (average of 7719 ± 2474 tags
339 per individual). After excluding individuals and SNPs with low coverage and removing SNPs
340 with low minor allele frequencies, the final genotype dataset contained 6067 SNPs scored
341 from 83 individuals, belonging to 10 populations, with a total genotyping rate of 0.919. All
342 populations had an average coverage $>70\%$ (Table 1). The population with the oldest
343 samples, Vila Real, only contained three individuals with high enough coverage, and was
344 therefore removed from population-level analyses, but the three individuals were retained in
345 individual-level analyses (STRUCTURE and LFMM).

346 *Adaptive genetic variation*

347 Bayescan identified 24 outlier SNPs potentially under selection. LOSITAN identified 224
348 SNPs as potentially under directional selection, which included 20 of the outlier SNPs also
349 identified by Bayescan. Allele frequencies in 13 outlier SNPs were significantly correlated
350 with either maximum temperatures (11 SNPs) or summer rainfall (10 SNPs). Significant
351 correlations were also identified within Iberia between five SNPs and maximum temperatures
352 and seven SNPs and summer rainfall (Table S2).

353 STRUCTURE assignment tests divided the full dataset into two main genetic clusters,
354 separating the English and Iberian samples. The Iberian cluster was further divided into two
355 clusters, separating the two northern populations, Bizkaia and Girona (Fig. S2). Therefore
356 LFMM was run with three latent factors for the full dataset and two for Iberia. LFMM
357 detected 93 outlier SNPs associated with maximum temperatures and 129 SNPs with summer
358 rainfall across the study area. In the Iberia-only dataset, 177 SNPs were associated with
359 maximum temperatures and 278 with summer rainfall. We identified eight SNPs potentially
360 associated with climate-adaptive genetic variation that were supported by all methods
361 (Bayescan, LFMM and logistic regressions; Table S3). The spatial distribution of genetic
362 variation in SNPs identified as potentially climate-adaptive indicates a lower frequency of
363 warm and dry adaptive alleles in the north and eastern Iberian populations (Bizkaia, Girona
364 and Valencia; Fig. S3; Table S4), and therefore these populations were classified as having
365 high adaptive sensitivity to climate change.

366 Genetic differentiation in SNPs identified as potentially associated with climate-adaptive
367 genetic variation was high overall (mean $F_{st}=0.245\pm 0.15$), but was substantially lower among
368 the north and eastern Iberian populations and among the southern and western populations.

369 Highest levels of differentiation were found between one English population (Devon) and
370 most other populations, apart from the north Iberian populations (Table S5). Genetic
371 differentiation in these climate-adaptive SNPs was related to environmental dissimilarity
372 between locations. Across the study area, genetic differentiation was correlated with
373 dissimilarity in both maximum temperatures (MRDM: $R^2=0.173$, $F=7.1$, $P=0.01$) and summer
374 rainfall ($R^2=0.137$, $F=5.4$, $P=0.023$), while within Iberia it was correlated with summer
375 rainfall ($R^2=0.228$, $F=5.6$, $P=0.028$; Fig. S4).

376 *Neutral genetic variation*

377 Genetic differentiation between populations based on the neutral dataset ranged between
378 0.024 and 0.106 (mean $F_{st}=0.056\pm 0.03$), with highest values between the English populations
379 and all Iberian populations (Table S4). Genetic differences between populations (Multilocus
380 AMOVA: $R^2=0.490$, $P<0.001$) and regions ($R^2=0.245$, $P<0.001$) were highly significant.
381 Differences between populations remained significant within Iberia ($R^2=0.279$, $P<0.001$).

382 Levels of neutral genetic diversity were lowest in the English populations, especially Dorset
383 (Heterozygosity=0.664). Iberian populations had generally high levels of heterozygosity, with
384 relatively lower levels in Granada (0.844) and Lisboa (0.885), and highest levels in Valencia,
385 Bizkaia and Valladolid (all >0.95). Relative neutral genetic diversity was ranked from low
386 (Heterozygosity <0.75) to medium (0.75-0.9) and high (>0.9) (Table S6).

387 **Determining range shift potential**

388 Genetic differentiation in neutral markers across the study area was positively related to
389 geographic distance (MRDM: $R^2=0.649$, $F=63.1$, $P=0.0001$) and to landscape resistance due
390 to decreasing habitat suitability, as measured by the ENM ($R^2=0.842$, $F=180.7$, $P=0.0001$),

391 decreasing forest cover ($R^2=0.588$, $F=48.6$, $P=0.0001$), increasing altitude ($R^2=0.299$, $F=14.5$,
392 $P=0.0004$) and increasing slope ($R^2=0.667$, $F=68.2$, $P=0.0001$). The ENM showed the
393 strongest correlations with genetic differentiation and was the only landscape variable that
394 remained significant after accounting for geographic distance ($R^2=0.197$, $F=8.3$, $P=0.005$;
395 Table S7). We obtained identical results when decreasing the resistance costs of dispersal
396 over sea to 120 (Table S8).

397 Under present conditions, all Iberian populations showed high levels of landscape
398 connectivity. Particularly high density of movement was predicted along the east coast of
399 Iberia, connecting the southern and north-eastern populations, and across the east-to-west
400 central axis of the peninsula. Overall density of movement was predicted to decrease under
401 future conditions, resulting in reduced connectivity between most populations and
402 geographical areas. In particular, eastern (Valencia), central (Albacete and Valladolid) and
403 southern (Granada) populations were predicted to become isolated, and were therefore
404 assigned low range shift potential. However landscape connectivity was predicted to increase
405 across the Pyrenees between the two northern populations and along the north Atlantic coast.
406 Movement out of Iberia appears to be limited both under present and future conditions, but
407 Iberia is predicted to become isolated under future conditions due to decreased habitat
408 suitability in southern France (Fig. 3).

409 **Identifying populations under threat**

410 When combining the effect of the three framework components we identified one Iberian
411 population (Valencia, east coast) at high risk due to high changes in climatic suitability (from
412 suitable to unsuitable and high increases in maximum temperatures and reductions in summer
413 rainfall), low frequency of SNPs identified as associated with climate-adaptive genetic

414 variation and limited future landscape connectivity. We identified two additional populations
415 in the central regions (Albacete and Valladolid) that are of medium-high risk because despite
416 high exposure to future changes and limited future connectivity, they have a relatively high
417 frequency of adaptive genetic variation and high levels of neutral genetic diversity. In
418 contrast, populations along the Atlantic coast (north and north-west of the peninsula) are
419 likely to be of lower risk due to more limited changes in climatic suitability and either high
420 future landscape connectivity or lower sensitivity (Table 6).

421 **Discussion**

422 We propose an applied integrated framework to identify wildlife populations under threat
423 from future climate change based on their extent of exposure to changing climatic conditions,
424 inherent sensitivity due to identified signatures of adaptive and neutral genetic variation and
425 range shift potential (Fig. 1). Our framework aims to address an important challenge
426 hampering conservation planning for species under climate change, the lack of inclusion of
427 measures of sensitivity and adaptive capacity in assessments of vulnerability, which currently
428 mainly focus on climate exposure (Butt *et al.* 2016). While previous studies discussed the
429 importance of including sensitivity and adaptive capacity when assessing climate change
430 vulnerability (e.g. Williams *et al.* 2008; Dawson *et al.* 2011; Pearson *et al.* 2014), this is the
431 first study to directly incorporate empirical genomic data to quantify sensitivity and assess
432 adaptive potential through the spread of adaptive genetic variation among populations.
433 Moreover, unlike previous studies, we consider intraspecific vulnerability and population-
434 level responses to global climate change, an important but often neglected research priority in
435 conservation biology.

436 Exposure to changing climatic conditions

437 To assess exposure to future climate change we combined ENMs with a comparison of
438 environmental dissimilarity between current and future conditions in key climatic variables
439 that are likely to affect bat survival and reproductive success. This helped identify priority
440 areas that are predicted to experience the greatest magnitude of change, the central regions
441 and the Mediterranean coast. However, it is important to note that apart from north and
442 northwest Atlantic coast areas, all Iberian populations are projected to experience maximum
443 temperatures outside the current thermal range of the species. Indeed, the entire Iberian
444 Peninsula is recognised as being under high threat from the effects of future climate change,
445 and Mediterranean ecosystems are predicted to experience the greatest biodiversity changes in
446 Europe due to the combined effect of climate and land use changes (Sala *et al.* 2000). In line
447 with previous studies (Razgour *et al.* 2013), the ENM analysis predicts range contractions for
448 *P. austriacus* across the southern part of its range, accompanied by expansion into more
449 northern latitudes. The inclusion of land cover variables and finer-scale resolution in the
450 ENMs resulted in less severe projections of future range losses in Iberia, but greater projected
451 losses in France, which will isolate the Iberian Peninsula.

452 Our assessment of exposure disregards the role of phenotypic plasticity or genetic adaptations
453 in enabling populations to persist in areas predicted to experience climatic conditions outside
454 the species' current environmental niche (Hoffmann & Sgrò 2011). However, evidence of
455 niche conservatism in climatic tolerance suggests that this species may be unable to survive in
456 climatically unsuitable areas in the future (Razgour *et al.* 2013).

457 Sensitivity due to adaptive and neutral genetic variation

458 Understanding adaptive genetic responses to environmental change in wild populations is
459 essential for biodiversity conservation under global change. Monitoring adaptive responses
460 can help identify populations and species that are not able to evolve fast enough to persist in
461 rapidly changing environments, and suitable donor populations that can help increase adaptive
462 potential through evolutionary rescue (Hansen *et al.* 2012). Yet even though it is recognised
463 that genetic variability is essential for the ability of species to adapt to environmental changes,
464 genetic components are often neglected in future climate change studies (Pauls *et al.* 2013)
465 and the genetic basis of evolutionary responses to climate change is still poorly understood
466 (Franks & Hoffmann 2012).

467 Advances in sequencing technologies have enabled genomic research on non-model
468 organisms and wild populations, and opened the door to identifying genetic features
469 underlying local adaptations, thus advancing our understanding of natural selection and
470 evolution (Hoban *et al.* 2016). However sequencing costs are still prohibitively expensive
471 when sampling a large number of individuals, particularly when a reference genome is not
472 available (Narum *et al.* 2013). Alternative approaches, such as ddRADseq, offer an affordable
473 way of obtaining a genome-wide perspective by targeting only a fraction of the genome,
474 rendering them particularly suitable for answering ecological and conservation questions
475 (Andrews *et al.* 2016). Such reduced representation techniques only sequence a small fraction
476 of the genome, and therefore only offer an indication of available adaptive genetic variation
477 (Lowry *et al.* 2017). Nevertheless, because RADseq provides a random sample of the genome
478 it is a powerful and efficient approach to study selection in natural populations and test for
479 evidence of adaptive differentiation and its geographical distribution (Catchen *et al.* 2017).

480 Bats have been the subjects of several recent genomic studies, shedding light on the evolution
481 of flight (Zhang *et al.* 2013) and echolocation (Parker *et al.* 2013). However this is the first
482 study to identify a signature of climate-driven selection in bats. By combining population
483 genomics and ecological approaches we identified eight SNPs representing genomic regions
484 that are potentially associated with climate-adaptive genetic variation. While genetic
485 differentiation in neutral SNPs was related to the effect of the landscape matrix on movement
486 between populations, differentiation in climate-adaptive SNPs was correlated with
487 environmental dissimilarity between locations, indicating a pattern of isolation by
488 environment as a result of local adaptations (Wang & Bradburd 2014).

489 Adaptation to local environmental conditions is thought to involve subtle changes in allele
490 frequencies because gene flow between populations can counteract local adaptations and the
491 fixation of adaptive alleles (Rellstab *et al.* 2015). These subtle changes, i.e. soft selective
492 sweeps, are harder to detect by genome scans for outlier loci, especially when selection has
493 not had sufficient time to substantially shift allele frequencies (Stapley *et al.* 2010).
494 Approaches that are driven by ecological hypotheses (genotype-environment association
495 analysis) are better able to detect ecologically relevant loci with small effects involved in
496 local environmental adaptations (Joost *et al.* 2013). Because only population genomic
497 approaches can detect complete selective sweeps, while ecological approaches are better
498 suited for detecting subtle changes, combining both approaches is essential for obtaining a
499 complete perspective on climate-driven genetic adaptations. Ideally, where possible, these
500 approaches should be combined with experimental testing and functional validation of fitness
501 or a trait in the absence of the putative adaptive alleles, though such validation is still
502 impossible for most experimental systems (Hoban *et al.* 2016).

503 Our framework focuses on an assessment of sensitivity to changes in climate based on
504 genomic data. Sensitivity can also be assessed using experimental evolutionary approaches.
505 Experimental studies measuring heritability of climate-related traits in various plant species
506 and *Drosophila* exposed to simulated climatic changes found that rates of evolution may be
507 too slow to match predicted rates of future climate change (reviewed in Jump & Peñuelas
508 2005). More recently, studies, primarily of plants, have combined genomic and experimental
509 approaches to identify local adaptations and genes under climate-driven selection based on
510 differential fitness of geographically diverse ecotypes raised under common garden
511 experiments (e.g. Fournier-Level *et al.* 2011; Savolainen *et al.* 2013). However, such
512 experimental approaches are not feasible for long-lived organisms with long generation time
513 and for many species of conservation concern, and results from such experiments may not
514 always be relevant for natural populations (Bailey & Bataillon 2015). Even in cases where an
515 experimental approach was applied to animals, field common garden studies have largely
516 failed to successfully incorporate fitness and genomic data (Savolainen *et al.* 2013).
517 Therefore, in our framework we focus on the genomic approach, but acknowledge that
518 sensitivity to climate change can be assessed using other approaches.

519 **Landscape connectivity and range shift potential**

520 Our framework applies primarily to relatively long-lived vertebrates with long generation
521 times and small population sizes, in which the rate of emergence and spread of novel adaptive
522 alleles in populations through de-novo mutations is likely to be too slow to respond to rapid
523 future climate changes (Hoffman & Sgrò 2011). Therefore, we emphasise the role of
524 landscape connectivity as an important component influencing the ability of populations to
525 respond to future changes through the spread of adaptive alleles between populations.

526 Through combining landscape genetics with ENMs we determined the effect of landscape
527 connectivity on movement patterns and the ability of *P. austriacus* to respond to climate
528 change by tracking changes to its environmental niche. We found that habitat suitability is the
529 main barrier to movement across the western part of this species' range. Using a predictive
530 landscape genetics approach, we showed how changing niche suitability is likely to limit the
531 future movement of individuals both within and out of Iberia. Species movement patterns are
532 not only a function of external factors like landscape connectivity, but also of internal factors,
533 like species' movement capacity (Nathan *et al.* 2008). The maximum recorded dispersal
534 distance in *P. austriacus*, 62 km (Riede 2001), is insufficient for individuals from most
535 Iberian populations to reach climatically suitable areas. Therefore range shifts are more likely
536 to be a gradual stepping stone process, involving the establishment of populations followed by
537 further dispersal events. This highlights the importance of the availability of suitable habitats
538 for range shifts in limited dispersal species.

539 Restricted future landscape connectivity will limit the movement of individuals between
540 populations and consequently reduce the rate of evolutionary adaptation to changing
541 conditions through reducing the spread of adaptive alleles among populations. Therefore
542 evolutionary rescue is unlikely without assisted translocation of individuals into populations
543 with a low frequency of alleles associated with warm and dry conditions. However even
544 under high dispersal or translocation scenarios, evolutionary rescue in spatially structured
545 populations may be impeded by local adaptations to heterogeneous environments that reduce
546 the fitness of migrants carrying climate-adaptive alleles (Schiffers *et al.* 2013). This further
547 strengthens the urgent need for an integrated framework to identify populations at high risk
548 and suitable donor populations based on patterns of adaptation to local environmental
549 conditions.

550 Conclusions

551 We developed an integrated framework to assess vulnerability to future climate change. We
552 demonstrate how our framework can be applied to vertebrates with relatively limited dispersal
553 abilities through combining genomic data with ENMs, spatial analysis and a predictive
554 landscape genetics approach under a risk assessment framework. Our study highlights the
555 importance of incorporating ecological and genomic data to predict both the sensitivity of
556 populations to future changes and their ability to shift their distribution to track changes in
557 environmental suitability. As evolutionary rescue in most vertebrates and species of
558 conservation concern is more likely to occur through the movement of individuals with
559 adaptive alleles between populations (Vander Wal *et al.* 2013), understanding movement
560 ecology and limits to future landscape connectivity is essential for predicting the ability of
561 populations to persist under climate change.

562 Assigned levels of threat can help prioritise and inform conservation action under climate
563 change. Conservation management can focus on either rescuing high risk populations
564 (through translocation of the entire population or of individuals with relevant adaptive
565 variation into the population) or increasing landscape connectivity to facilitate range shifts
566 and the spread of adaptive genetic variation to reduce threats to medium and medium-high
567 risk populations. As such our framework can contribute to transforming conservation
568 management under climate change from a crisis-driven response to more anticipatory and
569 predictive measures (Gillson *et al.* 2013).

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779 **Data Accessibility**

780 - The raw sequence data from this study have been submitted to the EBI European nucleotide
781 archive under project number PRJEB21291 (see Table S9 for barcode sample identifiers).

782 - SNP dataset in Genepop and Map/PED formats, STRUCTURE input and output files and
783 Maxent output files – Data available from the Dryad Digital Repository:

784 <http://dx.doi.org/10.5061/dryad.kv4g1> (doi:10.5061/dryad.kv4g1).

785 - R scripts used in the analysis: online Supporting Information.

786 **Author Contribution**

787 OR conceived and designed the study. KP and GJ advised on study design. OR, JJ, CI, HR
788 and AA collected the genetic samples. OR and JBT carried out the molecular lab work and
789 performed the data analysis. SM advised on data analysis. OR wrote the first draft of the
790 manuscript and all authors contributed to revisions.

791 **Table 1** – *Plecotus austriacus* populations included in the final genomic dataset with location,
 792 region, geographical area within the region, GPS coordinates (WGS1984), number of
 793 individuals and average population SNP dataset coverage.

Population	Year	Region	Area	Latitude	Longitude	Number of individuals	Average coverage
Lisboa	2013	Iberia	West	38.764	-9.250	10	91.2%
Bizkaia	2013	Iberia	North	43.331	-2.782	10	99.0%
Girona	2013	Iberia	North-East	42.323	3.166	9	95.0%
Granada	2013	Iberia	South	37.109	-4.170	8	85.0%
Albacete	2013	Iberia	Centre-East	39.296	-2.069	9	97.2%
Valladolid	2013	Iberia	Centre-North	41.581	-4.586	10	98.3%
Valencia	2013	Iberia	East coast	39.409	-0.960	9	98.6%
Vila Real	2009	Iberia	North-West	41.300	-7.800	3	73.4%
Devon	2011-2013	England	South-West	50.552	-3.550	8	90.3%
Dorset	2011	England	South-Centre	50.645	-2.315	7	71.7%

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796 **Table 2** – Variables and categories used to assess level of exposure to future changing
 797 climatic conditions. Formula indicates whether all variables were combined together or only
 798 one or two needed to be true. ENM refers to the outputs of the ecological niche model –
 799 continuous output for changes in relative occurrence probability, or binary output for changes
 800 in climatic suitability. Temperature and rainfall dissimilarity refer to differences between
 801 present and future (2070) conditions.

Level of Exposure	Formula	ENM	Temperature dissimilarity	Rainfall dissimilarity
1 (low)	ENM + Temp + Rain	- Change in relative occurrence probability <25% - Area remains climatically suitable	low: <6°C increase	low: <25% decrease
2 (medium-low)	ENM + (Temp OR Rain)	- Change in relative occurrence probability >25% - Area remains climatically suitable	medium: 6-8°C increase	medium: 25-50% decrease
3 (medium-high)	ENM OR Temp OR Rain	Area changed from climatically suitable to unsuitable	high: >8°C increase	high: >50% decrease
4 (high)	ENM + (Temp OR Rain)	Area changed from climatically suitable to unsuitable	high: >8°C increase	high: >50% decrease

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803

804 **Table 3** – Assessment of sensitivity based on the frequency of alleles identified as potentially
805 associated with climate-adaptive genetic variation in the population.

Level of Sensitivity	Mean frequency across all adaptive loci	No. adaptive alleles at frequency <0.25
Very high (++)	< 0.5	more than a third
High (+)	< 0.5	less than a third
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Low (-)	> 0.5	none

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807

808 **Table 4** – Integrating measures of exposure (Table 2), sensitivity (Table 3) and range shift
 809 potential to assess overall level of risk. Formula indicates whether all measures were
 810 combined together or only two needed to be true (Exp-Exposure, Sen-Sensitivity, Range-
 811 Range shift potential).

Risk Level	Formula	Exposure	Sensitivity	Range shift
Low	Exp + (Sen OR Range)	1	low (-)	high (+)
Low	Exp + Sen + Range	2	low (-)	high (+)
Medium	Exp + (Sen OR Range)	2	mid (0) - high (+)	low (-)
Medium	Exp + Sen + Range	3	low (-) - mid (0)	high (+)
Medium-High	Exp + (Sen OR Range)	3-4	high (+ / ++)	low (-)
High	Exp + Sen + Range	3-4	high (+ / ++)	low (-)

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814 **Table 5** – Changes in climatic conditions (Tmax=maximum temperatures, Rain=summer rainfall) and climatic suitability (based on the
 815 ecological niche model (ENM) predicted occurrence probability) for *Plecotus austriacus* populations in Iberia and England. Locations where the
 816 greatest changes are predicted to occur (>8°C increase in maximum temperatures, >50% decrease in summer rainfall and change from suitable to
 817 unsuitable areas) are highlighted in grey.

Population	Tmax (°C)	Rain (mm)	ENM (climatic suitability)	2070 Tmax (°C)	2070 Rain (mm)	2070 ENM	Change			
							Change Tmax (°C)	Change Rain (mm)	% Change Rain	% Change ENM
Lisboa	25.3	36	100	28.6	28	80	+3.3	-8	-22.2	-20.0
Bizkaia	23.6	225	38	29.9	120	40	+6.3	-105	-46.7	+5.3
Girona	25.6	159	76	32.1	90	7	+6.5	-69	-43.4	-90.8
Granada	30.0	45	80	36.9	39	37	+6.9	-6	-13.3	-52.5
Albacete	31.6	61	34	40.4	27	17	+8.8	-34	-55.7	-50.0
Valladolid	29.3	65	56	38.4	34	41	+9.1	-31	-47.7	-26.8
Valencia	28.5	87	44	35.6	42	5	+7.1	-45	-51.7	-88.6
Devon	19.8	171	90	25.5	107	95	+5.7	-64	-37.4	+5.6
Dorset	20.8	164	60	27.4	98	63	+6.6	-66	-40.2	+5.0

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820 **Table 6** – Identified level of risk to Iberian populations of *Plecotus austriacus* based on their
 821 extent of exposure to climate change (1=low; 2=medium; 3=medium-high; 4=high), overall
 822 sensitivity (+ high; 0 medium; - low), with sensitivity based on climatic adaptations and
 823 neutral genetic diversity in brackets, and range shift potential (+ high future connectivity; -
 824 low connectivity).

Population	Exposure	Sensitivity		Risk level
		(adaptive; neutral)	Range Shift	
Lisboa	1	- (- ; 0)	+	low
Bizkaia	1	+ (++ ; -)	+	low
Granada	2	- (- ; 0)	-	medium
Girona	3	0 (+ ; -)	+	medium
Valladolid	3	- (- ; -)	-	medium-high
Albacete	4	- (- ; -)	-	medium-high
Valencia	4	+ (++ ; -)	-	high

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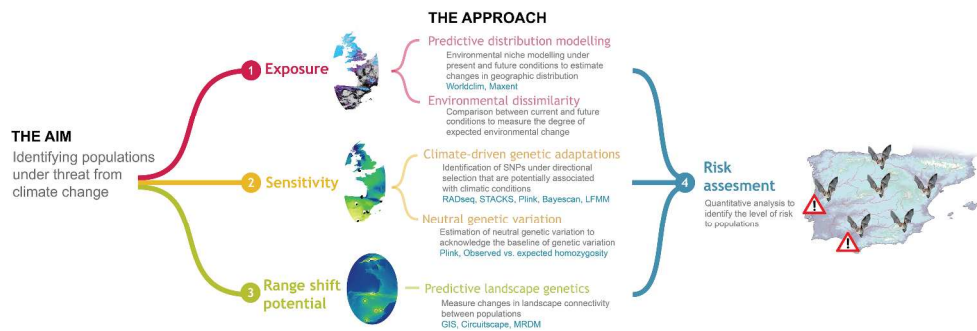
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827 **Figure captions**

828 **Figure 1** – The integrated framework to identify populations under threat from future climate
829 change, including the approaches and methods used to assess the different framework
830 components.

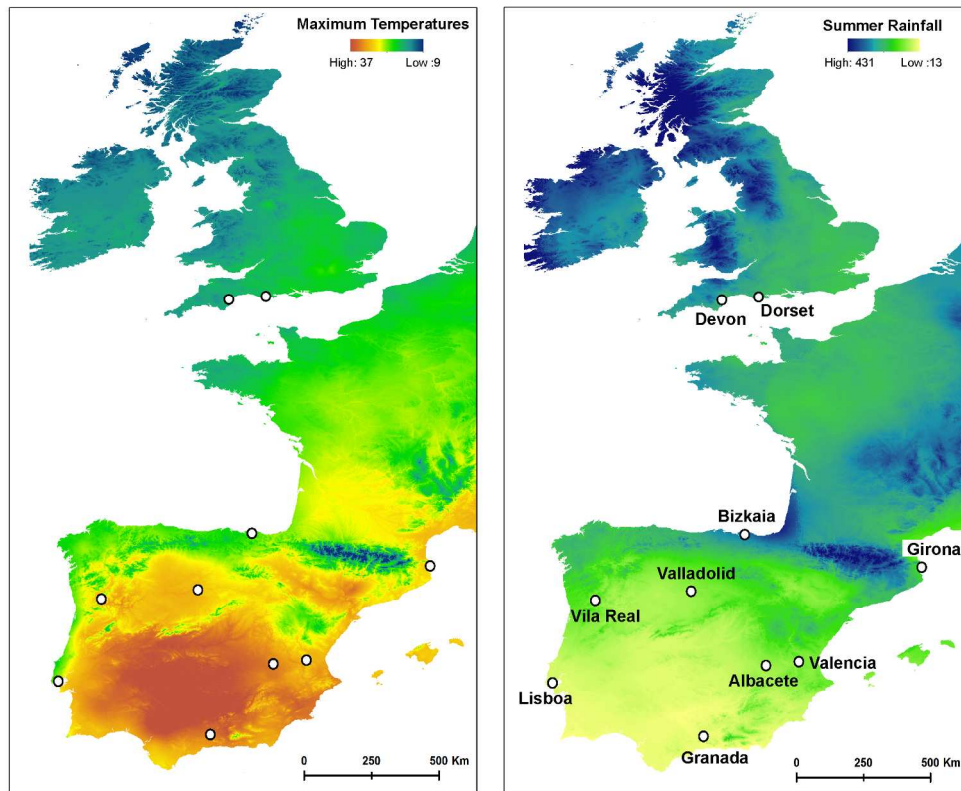
831 **Figure 2** – *Plecotus austriacus* populations included in the study presented over maps of
832 maximum temperatures and summer rainfall (www.worldclim.org).

833 **Figure 3** – Predicted distribution of suitable conditions for *Plecotus austriacus* based on
834 environmental niche models for present (A) and future (2070, B) conditions, and predicted
835 movement density maps between populations based on landscape resistance due to present
836 (C) and future (D) habitat suitability.



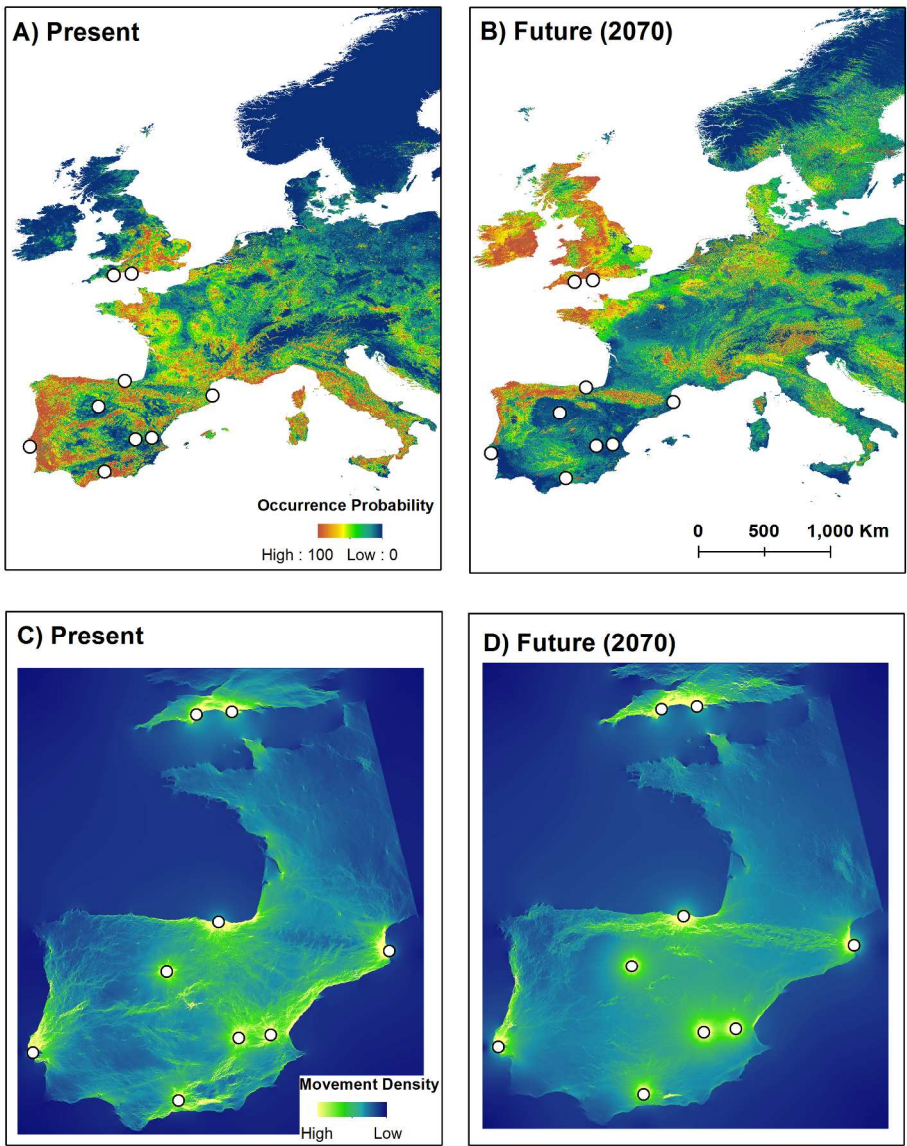
The integrated framework to identify populations under threat from future climate change, including the approaches and methods used to assess the different framework components.

942x300mm (299 x 299 DPI)



Plecotus austriacus populations included in the study presented over maps of maximum temperatures and summer rainfall (www.worldclim.org).

210x174mm (300 x 300 DPI)



Predicted distribution of suitable conditions for *Plecotus austriacus* based on environmental niche models for present (A) and future (2070, B) conditions, and predicted movement density maps between populations based on landscape resistance due to present (C) and future (D) habitat suitability.

209x266mm (300 x 300 DPI)

Table 1 – *Plecotus austriacus* populations included in the final genomic dataset with location, region, geographical area within the region, GPS coordinates (WGS1984), number of individuals and average population SNP dataset coverage.

Population	Year	Region	Area	Latitude	Longitude	Number of individuals	Average coverage
Lisboa	2013	Iberia	West	38.764	-9.250	10	91.2%
Bizkaia	2013	Iberia	North	43.331	-2.782	10	99.0%
Girona	2013	Iberia	North-East	42.323	3.166	9	95.0%
Granada	2013	Iberia	South	37.109	-4.170	8	85.0%
Albacete	2013	Iberia	Centre-East	39.296	-2.069	9	97.2%
Valladolid	2013	Iberia	Centre-North	41.581	-4.586	10	98.3%
Valencia	2013	Iberia	East coast	39.409	-0.960	9	98.6%
Vila Real	2009	Iberia	North-West	41.300	-7.800	3	73.4%
Devon	2011-2013	England	South-West	50.552	-3.550	8	90.3%
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Table 2 – Variables and categories used to assess level of exposure to future changing climatic conditions. Formula indicates whether all variables were combined together or only one or two needed to be true. ENM refers to the outputs of the ecological niche model – continuous output for changes in relative occurrence probability, or binary output for changes in climatic suitability. Temperature and rainfall dissimilarity refer to differences between present and future (2070) conditions.

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Population	Exposure	Sensitivity		Risk level
		(adaptive; neutral)	Range Shift	
Lisboa	1	- (- ; 0)	+	low
Bizkaia	1	+ (++; -)	+	low
Granada	2	- (- ; 0)	-	medium
Girona	3	0 (+ ; -)	+	medium
Valladolid	3	- (- ; -)	-	medium-high
Albacete	4	- (- ; -)	-	medium-high
Valencia	4	+ (++; -)	-	high