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A potential threat to amphibians in the European Natura 2000 network: Forecasting the distribution of the American bullfrog *Lithobates catesbeianus*

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ABSTRACT

Freshwater biodiversity is declining at an accelerated pace. Climate change and associated global warming and changes in precipitation patterns, combined with the expansion of generalist -invasive species are two of the main threats. Niche-based models (NBMs) are becoming inevitable tools in invasive species risk assessment and in conservation decision-making. *Lithobates catesbeianus* is an invasive species globally known for its adverse ecological impacts on native amphibians and biodiversity. To assess species current and future climatic suitable areas at the global and European scales we used an ensemble forecasting approach. We considered six climatic variables, three timeframes (current, 2050, and 2070), and two CO_2 emission scenarios. Temperature seasonality, minimum temperature of the coldest month, maximum temperature of the warmest month, and precipitation in the driest month were the most important variables predicting bullfrog occurrence. Globally currently 3.8% of land area is suitable for bullfrog and an increase of up to 5.2% in 2070 is expected. Increase in suitable areas is expected at higher latitudes, especially in North America and central Europe. Currently, 3.45% of total Natura 2000 area is suitable, and a predicted range gain of up to 355.93% (12.28%) is expected in the highest concentration scenarios predictions. This can indicate that the 64 native amphibian species present in the Natura 2000 network could be at increased risk. The choice of Natura 2000 for a geographic detailed analysis of the possible effects on native amphibians is due to its importance for habitats and wildlife conservation. Identification of its invasion-susceptible areas will allow resource and management practices optimization.

1. Introduction

Introduction and spread of invasive aquatic species (IAS) will increase as a consequence of global warming (Gama et al., 2017; IPCC, 2018). Climate change is expected to alter species' geographic ranges (Duan et al., 2016) and in a changing climate, species viability will be a reflection of their dispersal abilities (Velásquez-Tibatá et al., 2013). Species with poor dispersal abilities and narrow niches, such as many endemic species, will be more vulnerable to those changes (Malcolm et al., 2006) compared with good dispersers (Slatyer et al., 2013), such as invasive alien species. Most invasive alien species (IAS) will be able to follow their optimal temperatures across the landscape and outcompete slow dispersers, causing local extinctions (Urban et al., 2012).

The European Union (EU) has a strong nature protection legislation and implemented Horizon 2020, the biggest EU Research and Innovation programme, with nearly €80 billion of funding (European Commission - programmes). Resulting information, namely regarding climate change effects and invasive species presence, may be very useful for the sustained management of Europe's biodiversity, a very important goal for the EU.

Recent studies singled out amphibians as the most threatened vertebrate class with 32.5% of species likely to face a significant extinction risk (Stuart et al., 2004; Wang and Li, 2009). Three quarters of European amphibian species are endemic to Europe (Temple and Cox, 2009) and 64 of 85 European amphibian species are listed in the Annexes to the "Habitats" Directive (Council of the European Commission, 1992; Silva et al., 2009; Abéllan and Sanchéz-Fernandez, 2015). The Iberian Peninsula, Apennine peninsula, Balkan coast, and several Mediterranean islands host the greatest concentration of threatened amphibian species (Temple and Cox, 2009). An extensive review on 21 European amphibian species found that 90% were already negatively affected by climate change, exhibiting population declines and reduced survival rates, habitat suitability, and range sizes (European Environment Agency, 2016). From the survey performed by Abellan and Fernandez, 2015, those 64 amphibian native species in the Natura 2000 network are classified according to the global extinction risk

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status in the IUCN red list as LC: Least Concern – 37 species, NT: Near Threatened – 11 species, VU: Vulnerable – 10 species, EN: Endangered – four species, CR: Critically Endangered – one species; LC/NT – one species.

Protected areas are considered one of the most effective conservational strategies for biodiversity conservation (Bombi, 2010). Today, the most important conservation investment of the EU and world's most extensive network of conservation area is Natura 2000 network of protected areas (Araújo et al., 2006) comprising over 26,000 sites and 18% of the EU's land mass (European Commission, 2011). The network contains a significant proportion of remaining semi-natural habitats of high conservation value in Europe, which will be especially important as refuges for vulnerable species under changing climate (Gitay et al., 2002). 50 amphibian species (43 are endemic) are identified as species of European interest and are therefore covered by the Habitats directive (BISE, 2019). Climate change may reduce the relevance of the current protected areas for threatened species conservation (Hannah et al., 2007). SDMs can be useful tool to assess invasion risk and help in developing conservation strategies in such scenarios, e.g. addition of new protected areas. Also, a comparison between current and future areas of shared bioclimatic suitability and potential IAS-free refugia for threatened species can be made to help to prioritize the establishment of those new protected areas.

The American bullfrog, *Lithobates catesbeianus* is native to the eastern part of North America, from USA to Canada and has been introduced into approximately 40 countries on four continents, mostly via aquaculture and the aquarium trade (Lutz and Avery, 1999). Because of its widely recognized invasive character and adverse impacts on native species it has been considered one of the 100 worst invaders in the world (GISD, 2015). Currently it is recognized as a species of Union concern in the EU Regulation 1143/2014 on the prevention and management of the introduction and spread of invasive alien species. The preferred optimum environmental temperature of *L. catesbeianus* is 15–32 °C, with an average body temperature of 30 °C (Govindarajulu et al., 2006), being 38.2 °C the critical thermal maximum (Lillywhite, 1970) and egg development is impaired above 31 °C (Degenhardt et al., 1996). Bullfrogs may hibernate until late April and early May and breeding choruses develop with air temperature exceeding 20 °C and water temperature between 13 and 17 °C (Govindarajulu et al., 2006). Moore (1942) stated that embryonic bullfrogs have a high minimal temperature of 15 °C for development.

Adult bullfrogs are generalist predators eating any animal smaller than themselves (e.g. insects, crustaceans, fish, frogs, reptiles, birds, rodents, bats). The presence of other IAS, e.g. co-evolved fish and crayfish species, could also accelerate the impact of bullfrogs on invaded freshwater ecosystems and amphibian species present in them (Bissattini and Vignoli, 2017). Bullfrog directly impacts native amphibians through predation and competition for resources and indirectly through the spread of deadly amphibian pathogens such as the fungi *Batrachochytrium dendrobatidis* and *Saprolegnia ferax* (Nori et al., 2011). *B. dendrobatidis* is the agent of chytridiomycosis (Garner et al., 2006), an infectious disease that is considered one of the main causes of ongoing global amphibian decline and extinction and *L. catesbeianus* may be infected by the fungus without developing the disease (Daszak et al., 2004; Ficetola et al., 2007a). Currently, little is known about American bullfrog impact on native species in Europe. Introductions of the species have been observed in Belgium, France, Germany, Greece, Holland, Italy, Spain, and the United Kingdom and established populations detected in Belgium, France, Germany, Greece, and Italy. Three successful eradications were reported, two of them (UK and Germany) by killing of individuals (both adults and tadpoles) and complete drainage of ponds and a third (also in Germany), by fencing the pond and consequently killing of individuals (Ficetola et al., 2007a). Initial introductions were probably due to pet trading and further expansion of bullfrogs occurred due to natural dispersal (short distances), or secondary human mediated translocations such as commercial farming initiatives (Ficetola et al., 2007a). Nonetheless Ficetola et al., 2010 reported that for Italy, bullfrog dispersal did not reach its maximum due to habitat fragmentation, presence of barriers such as roads/urbanizations and changes in land use decreasing water availability (in ponds, wetlands, rice fields, etc). Invasion success depend on adaptability to local conditions and presence of adequate habitats (Ficetola and De Bernardi, 2004; Ficetola et al., 2007b).

Reported impacts on native species are sparse yet, the case of the threatened endemic Cretan frog *Pelophylax cretensis*, is a good indicator of how bullfrog expansion may affect native European species. In 2000, the bullfrog was introduced into Agia Lake, Crete, which resulted in the local extinction of *P. cretensis* (Adriaens et al., 2013). Also, Garner et al., 2006 reported that at least five bullfrog populations from France, Italy, and the United Kingdom are or were infected by the fungus *B. dendrobatidis* which may increase negative impacts over native amphibia populations.

Here, we modelled the potential global distribution of American bullfrog. The aims of this study were: 1) to determine the potential global distribution of *L. catesbeianus* with a special focus on Europe, as well as to assess the projected range change in potentially suitable areas across different time periods and greenhouse gas emission scenarios; 2) to identify Natura 2000 areas at risk of L. *catesbeianus* invasion under current and future climatic conditions and to discuss management actions that could be taken to tackle the threat.

2. Materials and methods

2.1. Species and environmental data

Individual records of 7124 worldwide native and invasive occurrence data of *L. catesbeianus* were collected from different archives, such as: GBIF (http://www.gbif.org/), HerpNet (http://www.herpnet.org/), speciesLink (http://www.splink.org.br/), EASIN (https://easin.jrc.ec.europa.eu/), IUCN (http://www.iucnredlist.org/), NA2RE (http://na2re.ismai.pt/). To describe the environmental conditions that might influence habitat suitability, six climatic variables were selected, with a cell resolution of 5 arc-min from the WorldClim datasets (Hijmans et al., 2005). A pairwise Pearson correlation was performed and highly correlated variables ($|r| > 0.80$) were excluded, to avoid collinearity in statistical models (Dormann et al., 2013). The climatic variables included in the model were: BIO4 = Temperature Seasonality, BIO5 = Max Temperature of Warmest Month and BIO6 = Min Temperature of Coldest Month, BIO13 = Precipitation of Wettest Month, BIO14 = Precipitation of Driest Month and BIO15 = Precipitation Seasonality, chosen from a set of 19 different climatic variables. Temperature variables describe the species' thermal tolerance, indicating upper and lower thermal limits, while precipitation variables are representative of water availability during the dry and wet periods of the year. Availability of permanent water bodies is critical for the American bullfrog, especially for tadpoles that overwinter in water and thus related to the likelihood of bullfrog population establishment (Liu and Li, 2009). The latest projected climate data of spatial resolution 5 arc-min (~10 km) were acquired from the climate model data used in the IPCC Fifth Assessment Report (IPCC, 2014), which holds information for both current and future conditions. Information on present-day distribution conditions (average 1950–2000) and projected climate scenarios for 2050 (average for 2041–2060) and 2070 (average for 2061–2080) were used and two contrasting representative concentration pathways (RCPs) were selected, RCP2.6 (rcp26)- stringent mitigation scenario and RCP8.5 (rcp85)- scenario without additional efforts to constrain emissions with very high anthropogenic greenhouse gas emissions, that is also called business-as-usual scenario (Pal and Eltahir, 2016). RCPs were used to quantify the lowest and highest gas emissions scenarios for each timeframe.

2.2. Modelling protocol

To model the potential distribution of *L. catesbeianus* at a global scale, we used an ensemble forecasting approach similar to the one used in Gama et al. (2016) and Gama et al. (2017). Algorithms are available in the BIOMOD2 package (Thuiller et al., 2009) in R software, version 2.14.0 (R Core Team, 2011). These included three regression algorithms [GLM (generalized linear models), GAM (generalized additive models), MARS (multivariate adaptive regression spline)], two classification methods [CTA (classification tree analysis) and FDA (flexible discriminant analysis)], three machine learning methods [ANN (artificial neural networks), RF (random forest for classification and regression), GBM (generalized boosted regression models)], and one climate envelope method [SRE (surface range envelope)]. Ensemble forecasting assumes that combined forecasts have a lower mean error than the individual forecasts constituting the ensemble (Araújo and New, 2007). Evaluation of individual models is performed by calculating a measure of central tendency and in our case, the median was used as it proved more by being less influenced by extreme output values (Araújo and New, 2007; Gama et al., 2017). Species occurrence data was coupled with an equal number of pseudo-absences randomly generated worldwide, since this has been shown to improve results in predicting the distribution of other freshwater invaders (Capinha and Anastácio, 2011) and to avoid biasing predictions towards more prevailing responses (Capinha et al., 2011). Coupled data was then separated into two datasets using 80% of the data to build a model, while retaining the remaining 20% for evaluating predictions (Thuiller et al., 2009). The true skill statistic (TSS) was calculated to evaluate model performance.

In the BIOMOD2 package, the importance of each variable is estimated through a randomization procedure (Thuiller et al., 2009). We assumed that the most important variables contributing to the model will be those with a relative importance above the mean of the predictor variables in the subsets (Allouche et al., 2006).

Five ensemble models were produced using a weighted approach based on TSS values, for the present, 2050 RCP2.6, 2050 RCP8.5, 2070 RCP2.6, and 2070 RCP8.5. To reclassify the resulting continuous maps into binary maps (unsuitable and suitable areas), the sensitivity-specificity equality approach (http://r-forge.r-project.org/projects/biomod/) was used, wherein the absolute value of the difference between sensitivity and specificity was minimized (Liu et al., 2005).

All binary suitability maps, for the present and the two future time frames (2050 and 2070) under two RCP scenarios (+2.6 and +8.5), were "reclassified" using ArcMap Spatial Analyst tools. Data (binary suitability maps) was analyzed in Arcgis and each of the important variables for the model was clipped using the suitable area as a "mask". In the resulting maps, the range of values for each variable were extracted, namely minimum, maximum and average. This was only done using the current suitability map, since for future projections what the program does is search for areas with similar temperature and precipitation values. The obtained suitability maps were also used as a "mask" to cut the Natura 2000 layer (https://www.eea.europa.eu/data-and-maps/data/natura-9/natura-2000-spatial-data/natura-2000 shapefile-1) and the percentage of intersected areas between binary and Natura 2000 maps were calculated.

3. Results

Table 1 shows mean values of TSS statistics and the respective standard deviation for each individual model used to compute the ensemble for current climatic conditions. Accuracy can vary between different models: with a mean of 0.907, Random Forest proved to be the best performing model, while BIOCLIM (SRE) showed to be the least predictive one (mean = 0.647). According to TSS (mean ± S.D. = 0.773 ± 0.017), mean accuracy was considered useful in predicting species distribution. Here, two out of nine models can be considered good to excellent, while the remaining models were classified as useful (Table 1).

Table 1
True skill statistic (TSS) for each of the algorithms (see Methods section) used to predict *Lithobates catesbeianus* distribution.

Algorithms	TSS	
	Mean	S.D
SRE	0.647	0.014
CTA	0.842	0.020
RF	0.907	0.029
MARS	0.774	0.013
FDA	0.746	0.015
GLM	0.721	0.012
GBM	0.799	0.011
GAM	0.766	0.012
ANN	0.753	0.023
Mean	0.773	0.017

Minimum temperature of the coldest month (BIO6) (importance: 0.346), maximum temperature of the warmest month (BIO5) (importance: 0.299), temperature seasonality (BIO4) (importance: 0.289), and precipitation of the driest month (BIO14) (importance: 0.284) were the most important predictors, having values of relative importance higher than the mean importance value (Fig. 1). Temperature seasonality ranges between 23 and 1154 with an average of 774. Temperature seasonality is the annual range in temperature expressed as the standard deviation *100. Maximum temperature of the warmest month ranges between 20 °C and 42 °C with an average of 30 °C. Minimum temperature of the coldest month for bullfrog suitability varies between −21 °C to 18 °C with an average of −4 °C. Precipitation values (precipitation of the driest month) vary between 0 mm to 160 mm, with an average of 48 mm.

Ensemble model performance was classified as excellent, based on the ensemble median TSS score of 0.895. The model correctly predicted 94.96% of *L. catesbeianus* presences (i.e. sensitivity) and 94.56% of its absences (i.e. specificity). Ensemble suitability models were ran for the present time (Fig. 2.a) and two future climate scenarios: 2050 and 2070. Binary (suitable–unsuitable) predictions were obtained using the sensitivity-specificity equality approach (Fig. 2.b). Ensemble modelling results indicate that 3.8% of corresponding global continental area was predicted to be suitable for *L. catesbeianus* under current conditions. When 2050 and 2070 climate scenarios were modelled, the percentages increased with elevating CO_2 emissions scenarios. The total continental area globally suitable for the American bullfrog increased to 4.76% and 5.01% for the 2050 projections and to 5.15% and 5.23% for the 2070 projections (Fig. 3), representing a maximum predicted increase in range change gain of 1.4%.

In total, the final ensemble model predicted that 2.3% of European continent and 3.45% of the corresponding Natura 2000 area is suitable for *L. catesbeianus* under current conditions. When future climate scenarios were modelled, maximum predicted range in Natura 2000 area rose 323.36% to 11.16% (RCP 2.6) and 262.12% to 9.04% (RCP 8.5) for the 2050 projections. In 2070 range increased 317.52% up to 10.95% (RCP 2.6) and 355.93% up to 12.28% (RCP 8.5) (Fig. 4). The percentage of suitable area in the Natura 2000 decreased when the higher RCP scenario was used to model species distribution in 2050 (comparing RCP2.6 and RCP 8.5); it was also higher in the lower RCP scenario for 2050 (RCP2.6) than in the lower RCP scenario for 2070 (RCP2.6).

4. Discussion

Since risk maps (e.g. Species Distribution Models - SDMs) visually describe where IAS may establish, they can be valuable tools for strategical IAS management planning. This work shows that climate change will favour the spread of *L. catesbeianus,* including in the European *Natura 2000* network of protected areas, therefore having great

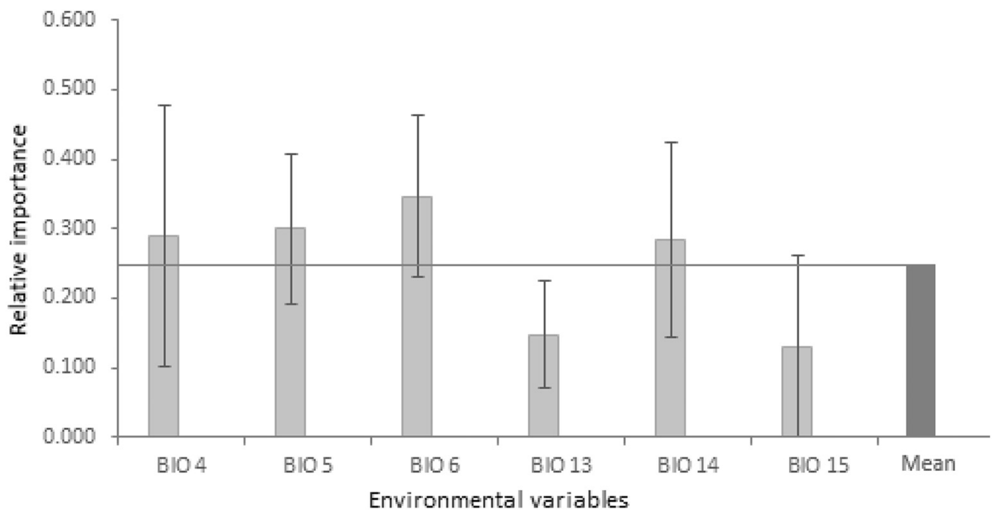

Fig. 1. Relative importance of the six environmental variables used to predict the distribution of *Lithobates catesbeianus,* with corresponding standard deviation values. The darker gray column and the horizontal line represent mean value of relative importance obtained from nine different modelling algorithms. Individual variables with relative importance above this horizontal line were assumed as important in determining L. *catesbaienus* suitability according to the models used. Environmental variables used were: BIO4 = Temperature Seasonality, BIO5 = Max Temperature of Warmest Month and BIO6 = Min Temperature of Coldest Month, BIO13 = Precipitation of Wettest Month, BIO14 = Precipitation of Driest Month and BIO15 = Precipitation Seasonality.

potential to threat native amphibian communities.

There are previous distribution models for *L. catesbeianus'* either globally or in South America, under the current climatic conditions (Giovanelli et al., 2008; Nori et al., 2011). As an example, Ficetola et al. (2007a) aimed to predict the current potential distribution of the bullfrog in Europe, but using data only from the native range to describe the bullfrogs' climatic requirements. However, using only native range presence data can strongly underestimate potential distribution areas, particularly for invasive species, whose anthropogenic dispersal can be very important (Mainali et al., 2015; Mau-Crimmins et al., 2006). Despite different modelling approaches, the predicted suitable areas under present conditions in Europe are similar to Ficetola et al. (2007a), especially in the Balkan and Apennine peninsulas. More specifically, the present conditions model predicted: global areas where the species has already been introduced (e.g. southern and south-eastern Brazil; Adriaens et al., 2013) and/or invasion is ongoing; (Allouche et al., 2006) areas in Europe where the species has been described in the literature, e.g. Belgium, France, Germany, Greece, Holland, Italy, Spain, the United Kingdom (Ficetola, Coïc, et al., 2007), and Slovenia (Kirbiš et al., 2016); (Araújo et al., 2011) the largest globally suitable area outside its native range (e.g. North America); and (Araújo et al., 2006) areas where the species has not yet been recorded globally (e.g. Bolivia) and European areas where the bullfrog has not yet been recorded (e.g. Croatia and Portugal).

Concerning amphibian biodiversity, the top five EU countries are: Italy, France, Spain, Germany, and Greece with 42, 38, 34, 23, and 22 amphibian species, respectively (Temple and Cox, 2009). Free-ranging populations of L. *catesbeianus* are also present in the majority of them, e.g. Italy, France, Germany and Greece (Ficetola et al., 2007a). Our model identified the Apennine peninsula and western Balkan coast (one of the areas with greatest concentration of threatened amphibian species in Europe (Red List of Threatened Species*,* www.iucnredlist.org; Temple and Cox, 2009) and central Europe as most vulnerable areas to future bullfrog expansion (Fig. 2.b).

With regard to global climate change scenarios modelling, which was never addressed before for this species, results show: that expansion of suitable areas occurs in all scenarios, increasing with time and RCP scenarios; (Allouche et al., 2006) expected expansion should be greater at higher latitudes (e.g. North America and throughout central Europe); and (Araújo et al., 2011) a tendency towards a decrease in suitable area in the Iberian Peninsula and south of the Balkan Peninsula. The predicted northern shift in distribution is in line with findings with other aquatic invasive species (Araújo et al., 2006; Banha et al., 2017; European Commission, 2013; Gama et al., 2017). Milder winters, extended spring and autumn seasons will make some areas more favourable for bullfrog, giving them opportunity to interact and impact species present in that areas. As dry conditions gradually increase in parts of Iberian and Balkan peninsula, they will become less suitable for the American bullfrog and other amphibians, since water is the key factor determining amphibian distribution in semi-arid regions (Araújo et al., 2006).

Changing climate will allow the American bullfrog to colonize protected areas in the EU more efficiently. Bullfrog range in Natura 2000 may raise 355.93% (2070 RCP 8.5 projection) when compared to the currently suitable areas 3.45% of Natura 2000 (Fig. 4.), reaching a 12.28% adequate area and the highest increase will also affect the areas with the greatest concentration of threatened amphibian species in Europe such as the Apennine Peninsula, and the western Balkan coast. Protected areas vulnerability to bullfrog expansion will reflect climatic conditions at the small scale changing as the climate does and will greatly depend also on their vulnerability to it. Degree of exposure, responsiveness, and adaptive capacity will define individual area vulnerability to climate change (Glick et al., 2011) and consequently, habitats that will be under stress will be even more vulnerable to invasive species.

A few studies suggested that for some locations (e.g. the Iberian Peninsula) national protected areas within the Natura 2000 network represented no more species of amphibians than expected by chance (Abéllan and Sanchéz-Fernandez, 2015; Lisón et al., 2017) when compared to other groups. One could argue then the little preponderance of this mechanism (Natura 2000) in protecting amphibians at risk. However, when species densities were included, an indication of the persistence of the species, Natura 2000 covered significantly more species (Abéllan and Sanchéz-Fernandez, 2015). Kukkala et al. (2016) indicated that the Natura 2000 network allowed to cover very high proportions of amphibian ranges as these have overall small ranges and that high rarity and species richness are concentrated in southern Europe, a high importance area within the Natura 2000 network. These results seem to corroborate the important role of the Natura 2000 network to protect endangered amphibian species, which could potentially be extended to other species, groups or areas/habitats at risk (Guareschi et al., 2020).

In accordance with the “Three-stage hierarchical approach” set by the Convention on Biological Diversity (UNEP, 2002), in highly suitable Natura 2000 areas where the species has not yet been recorded, focus must be on prevention. This means that the most important pathways of introduction must be controlled, and that early detection and rapid response protocols must be established. In areas where bullfrog has already been introduced and invasion is ongoing, the focus should be on minimizing impacts and stopping future spread. Eradication programmes have succeeded four times in Europe: one in the UK, two in Germany (Ficetola et al., 2007a) and one in Netherlands (Vane and

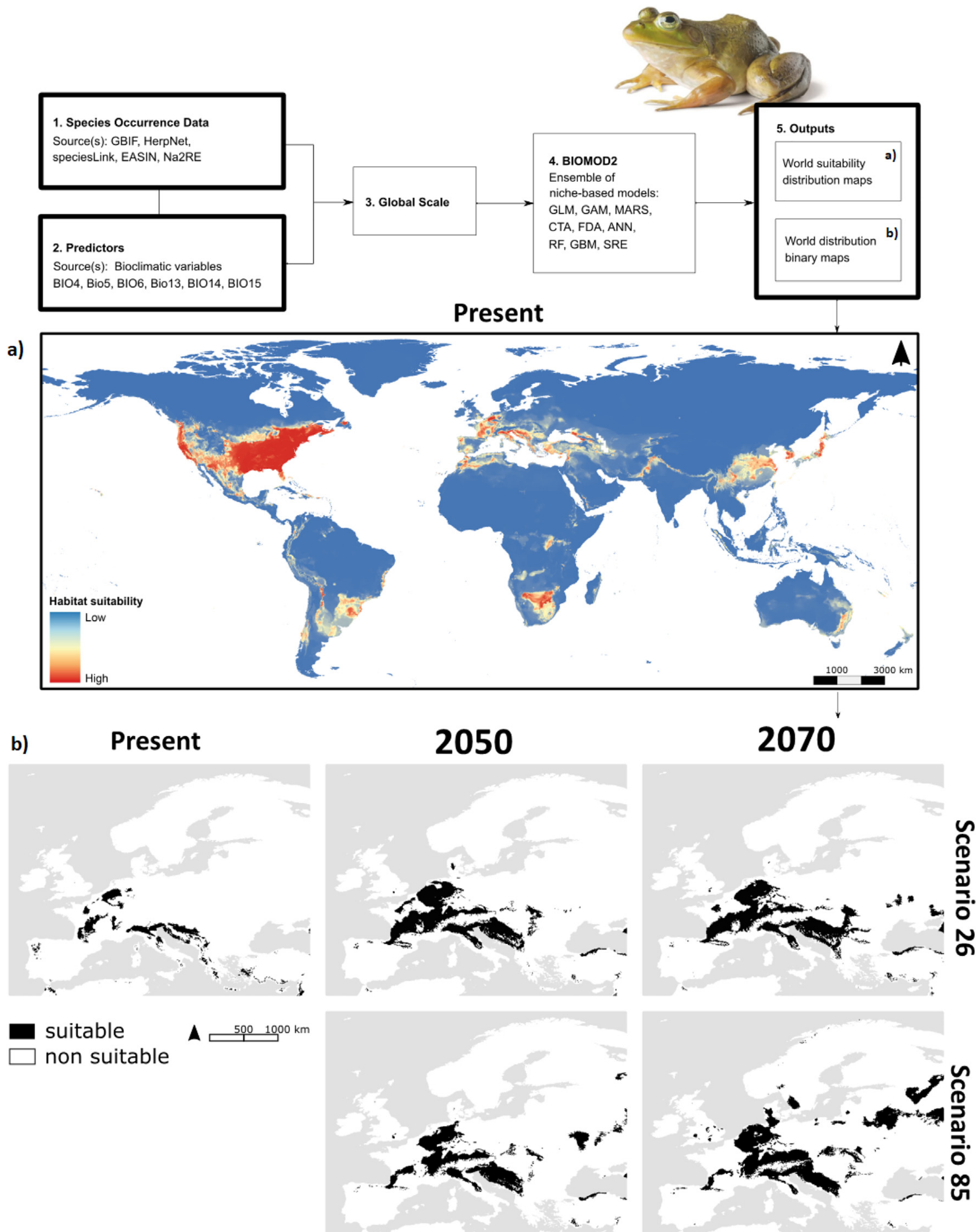

Fig. 2. Conceptual diagram of the study and a) Worldwide projection for environmental suitability of *Lithobates catesbeianus* at the present time; b) Binary maps (suitable–unsuitable areas) of predicted distribution of *L. catesbeianus* in Europe for three time periods (present time, 2050 and 2070) and two RCP scenarios (rcp26 and rcp85).

Runhaar, 2016). These programmes involved killing both adults and juveniles by draining ponds or fencing the breeding pond and killing all individuals early when populations were still small (Ficetola et al., 2007a). This indicates that similar rapid response protocols should be prepared for areas with high suitability, where the impact of bullfrog establishment would significantly affect present native species of high value for the EU. Currently there are ongoing programmes to control the populations of bullfrogs in Europe under several initiatives (e.g. Life CROAA, https://www.life-croaa.eu/en/actions/). The optimization and evaluation of the control methods will provide better recommendations on how to manage the bullfrog in nature and managed aquatic systems. Reintroduction of originally occurring native predators, e.g., pike, showed to be efficient methods for controlling larval bullfrog (Louette, 2012). Reintroduction of native predators is also an important step after eradication of invasive species, leaving ecosystem less vulnerable to reinvasion. New techniques showing the potential effectiveness of cold and pressure shock in reducing male fertility (Descamps and De Vocht, 2017) foster hope in the prospects of adopting the Sterile Male Release

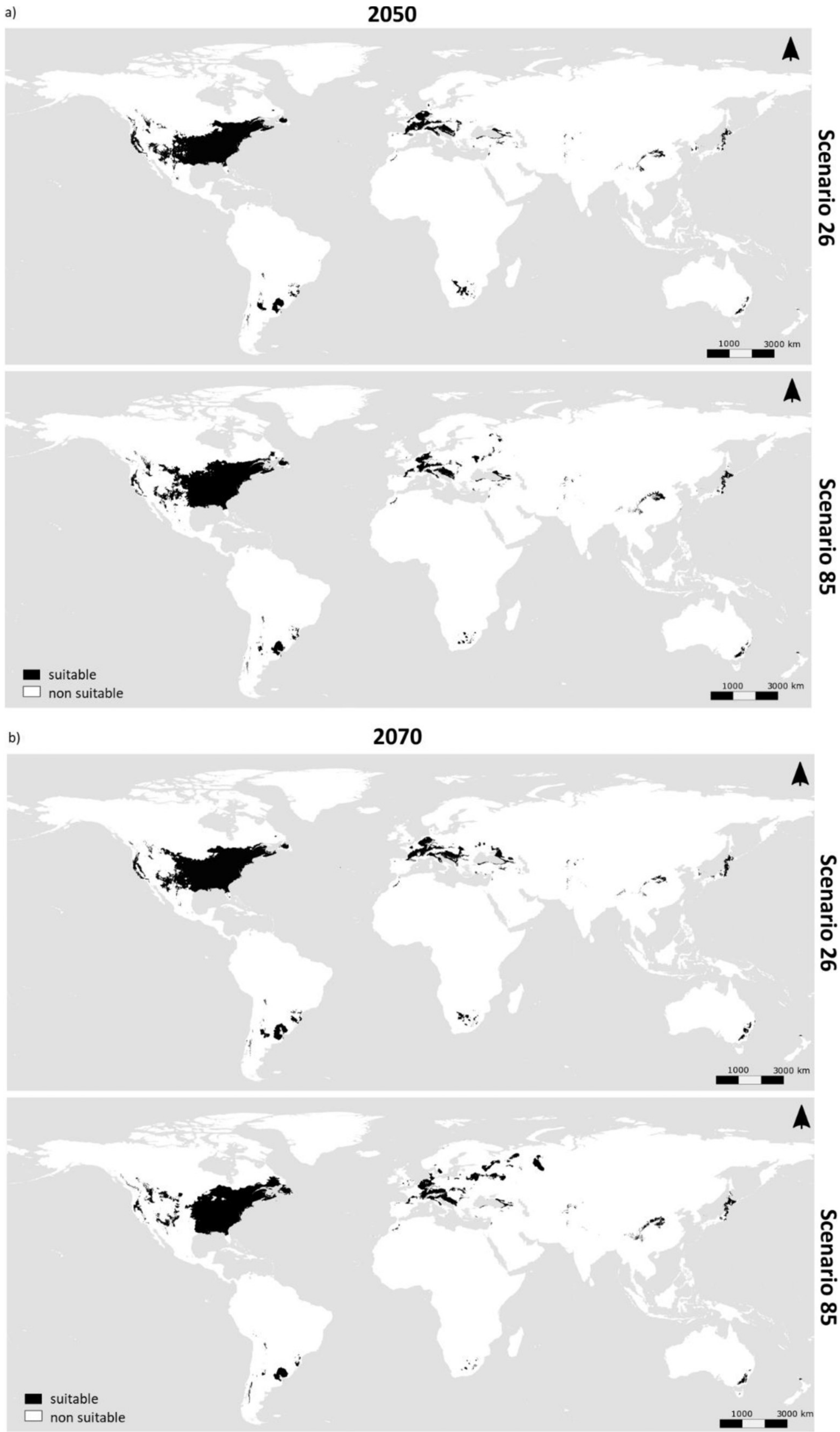

Fig. 3. Binary maps (suitable–unsuitable areas) of predicted distribution of *L. catesbeianus* for two time periods 2050 a) and 2070 b) and two RCP scenarios (rcp26 and rcp85).

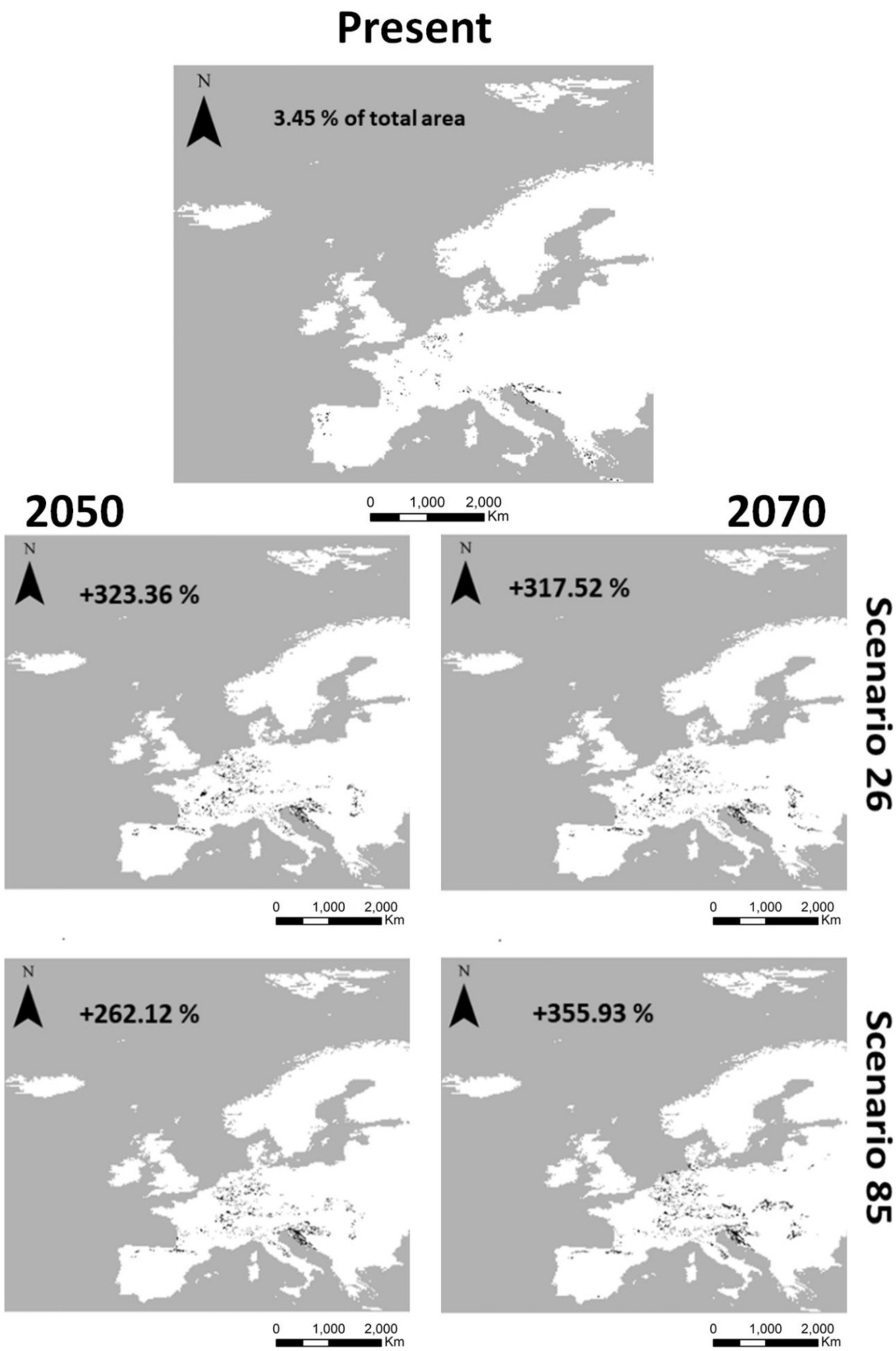

Fig. 4. Natura 2000 areas suitability for *L. catesbeianus* at present time and two future time frames (2050 and 2070) and two RCP scenarios (rcp26 and rcp85) with calculated percentages of change in suitability.

Technique (SMRT) to control bullfrog populations.

5. Conclusions

To conclude, this study showed changing climate will allow the American bullfrog to colonize Natura 2000 protected areas in the EU more efficiently, further spreading towards Northern and South-Eastern Europe, impacting already endangered native species. NBMs can help to understand the combined threat posed by climate change and invasive species on the distribution of threatened species. In this context we recommend particular attention to invasions by bullfrogs in the north of the Iberian Peninsula and throughout all Central Europe which are, according to IUCN, European areas with increased number of amphibian species per area.

CRediT authorship contribution statement

Iva Johovic: Conceptualization, Methodology, Data curation, Writing - original draft. **Mafalda Gama:** Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft. **Filipe Banha:** Conceptualization, Methodology, Formal analysis, Writing - original draft. **Elena Tricarico:** Conceptualization, Validation, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Pedro Manuel Anastácio:** Conceptualization, Validation, Formal analysis, Resources, Writing - review & editing, Supervision.

Declaration of competing interest

All authors certify that they have no affiliations with or involvement in any organization or entity that could be considered a source of conflict of interest in the subject matter or materials discussed in this manuscript.

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