

Electric circuit theory applied to alien invasions: a connectivity model predicting the Balkan frog expansion in Northern Italy

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Abstract. Invasive species are a major threat to biodiversity and alien herpetofauna already caused conservation problems in Italy and in the world. *Pelophylax kurtmuelleri* (Gayda, 1940) is a water frog native to Southern Balkans, that was introduced in NW Italy (Liguria) in 1941, from where it has spread to Piedmont, Lombardy and recently to Emilia Romagna. Surprisingly, during its expansion, the Balkan frog seemed not to be able to cross the Po River (with a single exception). So, we investigated the reasons for such directional limitation of range expansion, focusing on the potential role of the Po River. Combining ecological niche and connectivity models, we adopted a two-step process in order to assess: (i) if the habitat suitability differs between the two sides of the Po Plain; (ii) if the Po River could act as a barrier in terms of dispersal. Results showed that the northern side of the plain seems less suitable than the southern one, even if many suitable patches occur along the main left tributaries of the Po River and in the hilly part of the plain. The Po River behaves like a barrier for the Balkan frog, but the only known point on the north bank of the river indicates that it may be able to only slow the dispersal and not to completely stop it. So, the most probable interpretation for the absence of the Balkan frog from the Northern Po Plain, is the combination of habitat suitability and connectivity issues: the former makes less probable that new viable populations can establish in the North, the latter decreases the northwards flux of the frogs. Given these findings, the Balkan frog seems able to spread in the whole Northern Italy and the colonization of the northern Po plain could happen shortly.

Keywords. Balkan frog, *Pelophylax kurtmuelleri*, invasive alien species, connectivity models, Italy.

INTRODUCTION

Invasive alien species (IAS) are among the main threats to biodiversity at global scale (Clavero and García-Berthou, 2005), notably for freshwater ecosystems (Gallardo et al., 2016). Indeed, throughout a variety of interactions with the native biota (e.g., competition for space and resources, Carmona-Catot et al., 2013; predation on native fauna, Roy et al., 2012; genetic loss due to hybridization, Muhlfeld et al., 2014; spread of dis-

ease, Gherardi, 2006), IAS have been proved to have the potential to radically affect the invaded ecosystems. Herpetological examples of IAS are available (e.g., *Rhinella marina*, Shine, 2010; *Boiga irregularis*, Rodda and Fritts, 1992; Lever, 2003) and alien herpetofauna already caused conservation problems in Italy (e.g., *Trachemys scripta*; Ficetola et al., 2009; *Xenopus laevis*, Lillo et al., 2011; *Lithobates catesbeianus*, Federici et al., 2008; Ficetola and Scali, 2010; *Pelophylax ridibundus/kurtmuelleri*, Ficetola and Scali, 2010). As a consequence, the issue of IAS has

started to be listed among the most important threats to the conservation of the Italian herpetofauna (Scali, 2008).

Pelophylax kurtmuelleri (Gayda, 1940) is native to Greece, Albania, Serbia, and Macedonia. It was introduced in Italy in western Liguria region in 1941 for food purposes (Lanza, 1962). In the subsequent years, it spread in Liguria and Piedmont (Andreone, 1999). The species has been reported in several localities of Southern Lombardy between 2005 and 2010 (Razzetti et al., 2010). In 2016, the first occurrences for Emilia Romagna were reported (<http://www.ornitho.it/>; accessed on 15/01/2017). This invasive species may potentially threaten native water frogs (*P. lessonae* and *P. kl. esculentus*) through several negative interactions, as competition for habitat and trophic resources (Ficetola and Scali, 2010), hybridization (Ellstrand and Schierenbeck, 2000; Paganò et al., 2003), or increasing the spread of pathogens. Indeed, IASs have been proved to work as vectors (Garner et al., 2006) and the presence of the fungal pathogen *Batrachochytrium dendrobatidis* has been assessed in *P. kurtmuelleri* (Bellati et al., 2012). By combining these potential threats *P. kurtmuelleri* has the potential to negatively affect native amphibian populations and it calls for urgent and targeted studies on the real consequences of the introduction of this species in the Italian freshwaters. Beside this need, from a conservation perspective, the knowledge of possible expansion routes of this species could give a substantial benefit in planning future monitoring and control strategies.

Habitat connectivity has been recognized essential by both researchers and nature conservationists (Rödger et al., 2016) and maintaining it has become a crucial conservation strategy because it determines numerous ecological phenomena (McRae et al., 2016). For example, connectivity can mitigate the effects of climate change, by allowing species to shift habitat ranges in a rapidly changing climate (McGuire et al., 2016). Moreover, connectivity is fundamental for population survival in a fragmented landscape: isolation makes populations more vulnerable to processes such as inbreeding, genetic drift as well as demographic stochasticity, resulting in higher local extinction probabilities (Fahrig and Merriam, 1985; Taylor et al., 1993). However, the creation of ecological networks could be a double-edged sword, accelerating the spread and establishment of invasive species (Cowley et al., 2015). In this scenario, the dispersal of the Balkan frog in Italy seems to be a suitable case study for analyzing the potential effect of connectivity on the spread of an alien species. On the one side, the invasive range of this frog is expanding and every year several new records from previously unoccupied areas are reported ([\[www.ornitho.it/\]\(http://www.ornitho.it/\)\); on the other side, looking at the current distribution and at the past expansion of this species, it seems that the Po River is acting as a barrier, preventing the Balkan frog to colonize the northern part of the Po plain \(Fig. 1A\). Indeed, while being widespread South to the Po River, the species is almost absent northwards \(with the only exception of a single location in Travacò Siccomario \(PV\) at the confluence between Po and Ticino River, E. R. pers. obs.\). Nor its absence can be reasonably explained by a sampling bias, because i\) the species can be easily identified by its call; ii\) in this region many herpetologists and qualified naturalists usually work, iii\) the new technologies have made very easy to share data on animal occurrence \(e.g., \[ornitho.it\]\(http://www.ornitho.it/\), \[i-naturalist.org\]\(http://www.i-naturalist.org/\)\), and iv\) targeted field surveys in some areas of Piedmont and Lombardy failed to find the Balkan frog \(E.R. and S.S., pers. obs.\).](http://</p>
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Classical Biotic-Abiotic-Mobility theory for species distribution (Soberón and Peterson, 2005) predicts that a species occurs in a given place only if three main conditions are fulfilled: the area is appropriate in terms of the fundamental niche (habitat suitability), it meets the suite of biotic conditions necessary for the maintenance of viable populations (e.g., interspecific competition), and it has been accessible to the species over relevant time periods (connectivity). Following this framework and assuming that no major discrepancies occur in the herpetological communities on both sides of the Po Plain (Bernini et al., 2006), we might interpret the absence of the Balkan frog as the consequence of (i) the lack of suitable areas to the North of the Po River (abiotic factors hypothesis) or (ii) the barrier effect of the Po River itself, which constitutes an almost impermeable obstacle to the northwards dispersal (movement factor hypothesis). So, the present study aims at (i) assessing if the habitat suitability differs between the two sides of the Plain, supporting the abiotic factor hypothesis, and, if not, (ii) evaluating if the Po River could be an almost impermeable barrier in terms of dispersal, according to the movement factor hypothesis.

To answer these two questions we used a combination of both Ecological Niche Models (ENMs; *sensu* Silero, 2011) and Connectivity Models (CMs): the former allows us to compare the two sides of the plain in terms of availability of suitable habitats; the latter let us testing which level of Po River permeability may translate into an effective reduction in the availability of northwards dispersal routes. Notably, CMs were obtained applying the electrical circuit theory (McRae, 2006; McRae et al., 2008) to the landscape where a species occurs. In this framework, the landscape is treated as a conductance surface where each element (cell in a raster) is linked to

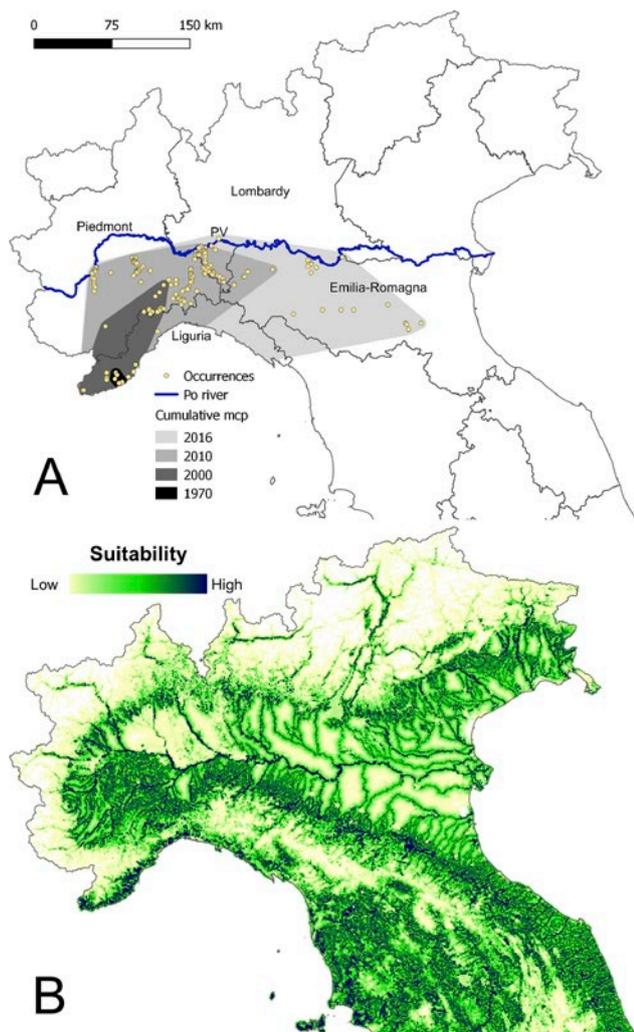


Fig. 1. (A) Map showing the study area and the range expansion of *Pelophylax kurtmuelleri* from the introduction to 2016. Polygons represent the invasive range of this species at different points in time. Dots represent occurrence points. PV: occurrence locality north of the Po River; (B) habitat suitability map for *Pelophylax kurtmuelleri*.

its neighbors and has its own conductance (i.e., permeability to current flow). Occurrences become the points in the network where current enters or exits the circuit (McRae, 2006), and current intensity across the landscape measures the animal potential flux (McRae et al., 2008). The outcomes are current maps representing the most probable connection pathways among suitable areas (McRae, 2006; McRae et al., 2008). Since this theory was introduced in the ecological field, this approach has been used for a wide range of purposes, including the analysis of IAS (Cowley et al., 2015).

MATERIAL AND METHODS

Occurrence data

The occurrence data of *P. kurtmuelleri* from its invasive range in Italy were obtained from different sources: historical published records, authors' personal observations, and records by experienced herpetologists (see acknowledgments for a full list). All locations (Fig. 1A) were georeferenced on a 1×1 km grid. The study area was delimited to Northern and Central Italy (Fig. 1A). Since records of Balkan frog from new localities are reported almost every week we decided to stop the data collection at the end of 2016.

Ecological niche model

ENM was fitted using MaxEnt (Phillips et al., 2004)(Phillips et al., 2004), version 3.3.3k (MaxEnt, 2017), which have proved to outperform other methods based on presence-only or presence/background data (Elith et al., 2006). A set of nine raster maps at a 1×1 km resolution were arranged in order to account for ecologically meaningful factors: distance from streams, land cover (i.e., the relative coverage inside each 1×1 km cell for each land cover class) and elevation (Table 1). All environmental variables were derived from maps freely available on the web (ISPRA, 2017): a vector layer of the Italian hydrographic network, a raster layer of the land use (Corine Land Cover) referred to 2012, at 100×100 m resolution, and a digital elevation model at 75×75 m resolution. To take into account multicollinearity among predictors (which can lead to model overfitting; Warren and Seifert, 2011), the variance inflation factor (VIF) was obtained for each variable and used as diagnostic statistic: values around one indicate no substantial problems, while the more they depart from one, the more the multicollinearity is serious (e.g., a value of 10 represents an unacceptable value; Faraway, 2004). Since the variable "notirri" (not irrigated crops; Table 1) showed a high VIF (7.80), this predictor was removed. Conversely, all the other predictors showed low multicollinearity (Table 1) and had low bivariate correlation coefficients (Supplementary material S1).

The availability of environmental factors was determined on the basis of 10 000 background cells randomly drawn from the whole study area. HSM performance was assessed using a cross-fold validation procedure considering the area under the receiver operating curve (AUC) as performance score (Merow et al., 2013): the occurrence dataset was randomly split into ten equal parts; each of them was used as the test dataset for the model built with the remnants nine. Accordingly, all records were used nine times for training and one for testing the model, and all the observations are equally weighted in the training and in the testing of the model (Merow et al., 2013). The final HSM was obtained by averaging the prediction of the ten ENMs from each replicate. In order to avoid overfitting, MaxEnt was run allowing only "linear", "product" and "quadratic" features (Warren and Seifert, 2011). Data preparation and modeling were carried out in R environment (R Core Team, 2017), using the raster (Hijmans, 2016) and dismo (Hijmans et al., 2015) packages.

Table 1. List of the environmental variables considered in building HSM. “Source” refers to the original map from which the data were derived or drawn. VIF (variance inflation factor): the VIF values for the whole set of variables (before) and for the subset obtained eliminating “notirri”, the most collinear variable (after).

Name	Description	Range	Source	VIF	
				before	after
dem	Average altitude	1-4180 m	digital elevation model	1.51	1.55
streamdist	Minimum distance from a stream	0-37000 m	streams vector	1.13	1.11
irri	Percentage coverage of irrigated fields	0-100%	land use	1.57	1.04
notirri	Percentage coverage of non-irrigated fields	0-100%	land use	7.80	removed
open	Percentage coverage of meadows	0-100%	land use	1.17	1.01
shrubs	Percentage coverage of bushes	0-100%	land use	2.19	1.44
urban	Percentage coverage of urban areas	0-100%	land use	2.17	1.12
wetlands	Percentage coverage of wetlands	0-100%	land use	1.30	1.02
woods	Percentage coverage of woodlands	0-100%	land use	4.68	1.28

To compare the suitability of the northern and southern sides of the Po Plain, we computed a kernel density estimations of the distribution of suitability values: we firstly cut HSM with a 20 km buffer around the Po River, and then we fitted and graphically compared two separate Gaussian kernels with fixed bandwidth (0.03; obtained using the native R function “density”) for the North and South riversides.

Connectivity models

CMs were built using Circuitscape (Shah and McRae, 2008), version 4.0.5 (Circuitscape.org, 2017), a software designed to model habitat connectivity using the electrical circuit theory (McRae, 2006; McRae et al., 2008). Conductance maps were derived from HSM by rescaling it between 0 (minimum suitability) and 1000 (maximum suitability), in order to reduce computational time due to floating points values. Further, to better fit the known ecology of the species, which is never found above 800 meters a.s.l., in both its native and invasive range (Sacchi et al., 2011), the cells at higher elevation were considered absolute barriers. For this purpose, these cells have been assigned an NA value, completely excluding them from the CMs computation. This modified suitability map was used as the conductance map for the reference model (CM_{ref}), i.e., the model that considers the suitability as the only parameter affecting the potential spread. To evaluate the barrier effect of the Po River, six more CMs were built, by forcing the conductance of the cells intersecting the Po River at increasing values: 0 (absolute barrier, CM_0), 1 (minimum permeability, CM_1), 10 (CM_{10}), 50 (CM_{50}), 100 (CM_{100}), and 500 (CM_{500}).

Circuitscape was run in advanced mode (McRae et al., 2013) to allow for the use of arbitrary current sources and grounds: all the occurrence cells were set as current sources, while the cells at the perimeter of the study areas represented the ground. So, the model was able to predict the possible spread of this invasive species considering all possible directions in one fell swoop (Cowley et al., 2015). When multiple

sources are set simultaneously, a possible “repulsive” effect with no ecological basis can be observed. Indeed, current flows from the sources (positive) to the ground (negative) and is prevented from moving between two sources (positive poles). To avoid this problem Circuitscape was run separately for each source cell while leaving ground unchanged. Then, the final model was created by cumulating all the current maps obtained in this way. For this purpose, a custom R function was developed (Supplementary material S3).

To test possible changes of the predicted spread under different Po permeability scenarios, each out of the six connectivity maps was compared to the CM_{ref} (i.e., the map obtained without manipulating the Po permeability). The comparison was made using Map Comparison Kit software, version 3.2.3 (MCK, 2017), a tool specifically developed for understanding the nature and the spatial distribution of the differences between map pairs (Visser and de Nijs, 2006). The software allows for a pixel-by-pixel comparison, whose outcome is, in turn, a map highlighting the location of the differences. Further, it computes an overall similarity index between a map pair: zero stands for two completely different maps, one stands for an identical pair (Visser and de Nijs, 2006). Given that CMs are numerical, the “fuzzy numerical” algorithm was applied (Visser and de Nijs, 2006).

RESULTS

Ecological niche model

A total of 299 occurrence points were collected (corresponding to 160 occupied cells). The ENM had a good performance ($AUC = 0.807 \pm 0.012$; mean \pm standard error), with the highest variable contribution of altitude ($43.6 \pm 0.4\%$), followed by distance from the nearest stream ($27.1 \pm 0.6\%$). The response curves showed that suitability scores decrease with increasing altitude and

distance from stream (Supplementary material S2). Suitability areas crossed the whole study area (Fig. 1B), closely matching the hydrographic network: the suitability increases where the network is dense, the opposite where it rarefies. On average, the southern side of the Po River appeared more suitable than the northern one, where many unsuitable areas can be found just next to the riverside (Fig. 1B). Indeed, the suitability distribution of the two sides of the Po River showed a North-South asymmetry (Fig. 2): the southern side has more high-scoring cells than the northern one, which, in turn, still maintains some highly suitable areas. The distribution of the observed occurrences agreed with the HSM to the South, but not to the North of the Po River.

Connectivity models

The reference connectivity model (CM_{ref} , Fig. 3A) depicted a situation where the current was able to flow both southwards and northwards of the Po. By manipulating the permeability, the obtained CMs behaved in two sharply different ways (Fig. 3): when the Po River was set as an absolute barrier (CM_0 , Fig. 3B), current mainly flowed southeast, and only a very small amount of current flowed in the northern side of the map; high current flow was visible along the entire coast of Liguria up to Northern Tuscany and also starting from Southern Lombardy across all Emilia Romagna, reaching Northern Marche region. On the opposite, as soon as the Po permeability was increased (CM_1 , CM_{10} , CM_{50} , CM_{100} , CM_{500} ; respectively from Fig. 3C to Fig. 3G), the northern part of the map suddenly matched CM_{ref} with current flowing along all the main Po's tributaries such as Dora Baltea, Sesia, Ticino, Lambro, Adda, Serio, Mincio, and reaching also other major rivers like the Adige. This convergence could be already observed in CM_1 (Fig. 3C), i.e., the model corresponding to the lowest permeability scenario.

Differences and similarities were confirmed by the fuzzy numerical comparison performed in MCK: similarity maps (Fig. 4) showed nearly no differences between CM_{ref} and maps where Po is not set as an absolute barrier (CM_1 to CM_{500} ; from Fig. 4B to Fig. 4F), while the comparison between CM_{ref} to CM_0 (Fig. 4A) lead to significant differences in the northern part of the map. The rapid convergence towards CM_{ref} was noticeable considering the sudden growth of the overall similarity indices among CM models and CM_{ref} (Fig. 5): when Po permeability increased from 0 to 1 (i.e., the minimum allowable permeability value) the similarity index rose from 0.36 to 0.97.

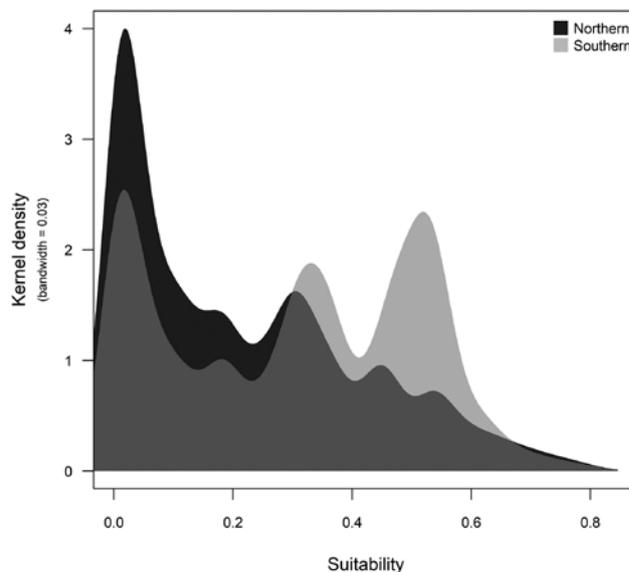


Fig. 2. Comparison of the available suitability of the northern and southern side of the Po River. Density estimation was restricted to a 20 km buffer (to the left and to the right of the river) and a fixed bandwidth (0.03) was used in both cases.

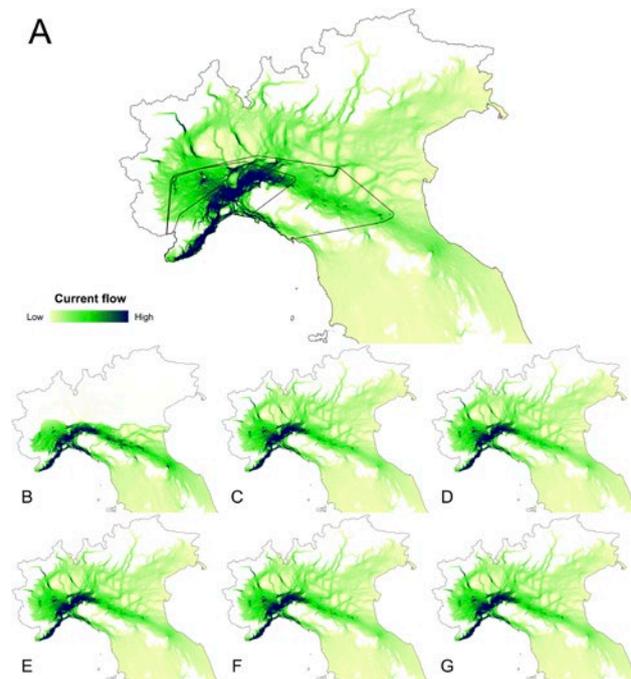


Fig. 3. Connectivity models showing the current flow at the variation of Po permeability. (A) Reference model without any modification to Po permeability CM_{ref} . Polygons represent the temporal range expansion (see Fig. 1A); (B) Po as an absolute barrier CM_0 ; (C) permeability set at minimum value of one CM_1 ; (D) permeability set at 10 CM_{10} ; (E) permeability set at 50 CM_{50} ; (F) permeability set at 100 CM_{100} ; (G) permeability set at 500 CM_{500} .

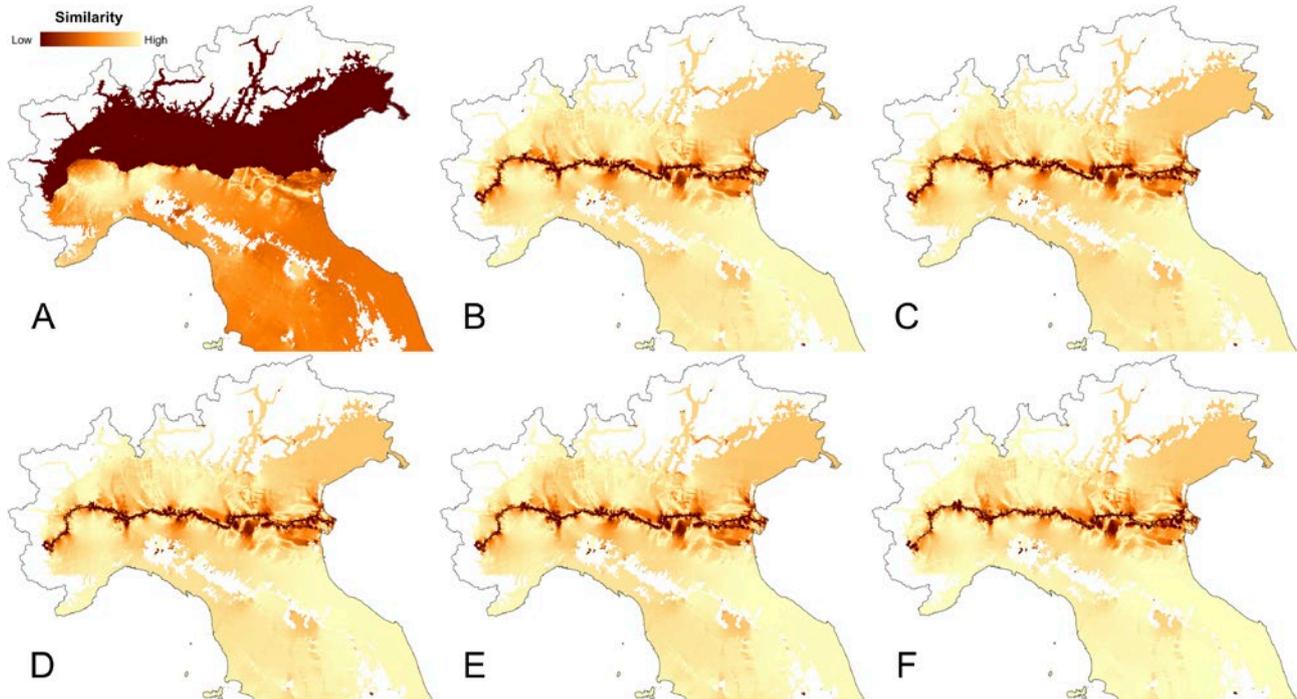


Fig. 4. Similarity maps of the fuzzy numerical comparison performed in MCK. Comparison between (A) CM_{ref} and CM_0 , (B) CM_{ref} and CM_1 , (C) CM_{ref} and CM_{10} , (D) CM_{ref} and CM_{50} , (E) CM_{ref} and CM_{100} , (F) CM_{ref} and CM_{500} .

DISCUSSION

The establishment of the Balkan frog in Italy started in 1941 from western Liguria (Lanza, 1962). Since then, the species is still spreading over Northern Italy, even it has not been actually able to occupy the northern side of the Po plain. HSM showed that the northern plain offers less suitable areas than the southern side, at least within the 20 km buffer zones along the riverbed (Fig. 1B and 2). At the same time, CMs pointed out that the river may act as a barrier itself, preventing the species to reach the northern corridors (i.e., the left-side tributaries of the Po River, Fig. 3). Together, these findings suggest that the absence of the Balkan frog from the North Po Plain may be due to the combination of these two factors, rather than to a prevalent effect of one of them. Indeed, the abiotic factor hypothesis is only partially supported by HSM. According to the known ecology of the species, suitable areas include lowland and hilly sites, near to streams and rivers, regardless of the habitat types (Sacchi et al., 2011). Even though these patches are more common and dense South to the Po, they undoubtedly occur also on the opposite riverside. Similarly, the movement factor hypothesis is fully supported only when the Po River is set as an absolute barrier (CM_0 , Fig. 2B). Even a minimum permeability would result in a widespread inva-

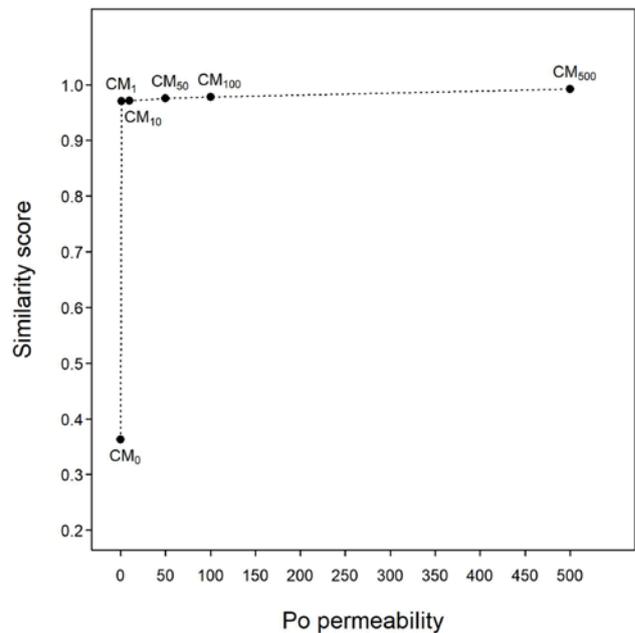


Fig. 5. Growth of the similarity index among CM models and CM_{ref} over the increase of Po permeability.

sion of the Northern Italy through the main tributaries of the Po River. Further, the occurrence of a record north to the Po River (PV in Fig. 1A), makes the absolute barrier hypothesis unrealistic. Assuming that the record was not related to anthropogenic transport, it would indicate that the Balkan frog actually keeps the potential of crossing the Po, even though very limited. Consequently, the lack of records of this species in the North may be due to a co-occurrence of both connectivity and suitability constraints, i.e., the barrier effect of the Po River and the spatial structure of the suitable habitat patches in the northern Po plain.

While river banks are often suitable habitat for many amphibians, the river itself could act as a biogeographical barrier for several reasons, such as large size of the riverbed (Lampert et al., 2003; Li et al., 2009; Geissler et al., 2015); therefore, the Po River might have played a role in mitigating the colonization of the north bank. Furthermore, the spatial distribution of suitable habitat patches may also have played a significant role. As mentioned above, suitable patches are more common and denser south to the Po, while northwards they concentrate along the major Po tributaries, which head north-south (Fig. 1B). In this context, colonization of new habitat patches can happen only if migration of individuals occurred in northern direction. Combining these two phenomena, the result is that the gap between the northern and southern distribution of Balkan frog may not be there in the future, and the colonization of the northern Po plain is just a matter of time. In this scenario, once colonized the major corridors (i.e., Alpine tributaries, such as Dora Baltea, Sesia, Ticino, Lambro, Adda, Serio, and Mincio, as well as other major rivers like the Adige), the Balkan frog could easily spread in the whole northern Italy.

In the last years, connectivity modeling became a widely used tool to study connectivity among populations, either for conservation purposes or to study species movement (Chetkiewicz et al., 2006; McRae et al., 2016). Until now, electrical circuit theory has been applied to ecology mainly to study connectivity in a network of habitat patches already occupied by native species and only one study focused on modeling the spread of alien species (Cowley et al., 2015). In our study, we showed how connectivity models could be a useful tool to deal with alien invasive species. We are aware that a number of points have to be improved. Habitat selection applies both to residency and movements (Chetkiewicz et al., 2006) and for this reason HSM has been recently used for deriving landscape connectivity (Rödger et al., 2016). However, the use of habitat suitability as a proxy for landscape connectivity has been recently criticized, because animals might be able to move through less suitable are-

as, particularly during long-distance movements (Keeley et al., 2017). Furthermore, hybrid models that consider population dynamics should allow to better understand the processes driving biological invasions and make more reliable predictions (Gallien et al., 2010). Anyway, connectivity models and Circuitscape software in particular, are still useful for conservation and management related to alien species, and they can return information on where to intervene to try to contain the phenomenon. About northern Italy invasion by Balkan frog, our conclusions suggest two possible scenarios: i) Po River is an absolute barrier and is preventing the Balkan frog from spreading in the northern Italy; ii) Po River is not an absolute barrier and is only mitigating the Balkan frog's breakthrough. So, where should conservation efforts be focused? If Po is an absolute barrier, our suggestion is to understand which features play a role in this mechanism (e.g., permanent water, large riverbed; Lampert et al., 2003), in order to take advantage of those characteristics by preserving or reinforcing those features, thus avoiding the northern Italy invasion. If Po River is not an absolute barrier, the situation would be much more critical and the only tool currently at our disposal might be the monitoring of the phenomenon, focusing the effort on the mayor corridors identified by this work (i.e., left Po tributaries) and on the northern side of the plain.

What research and nature conservation policy will be able to do in the future will be fundamental to preserve the environment from this invasion. Despite several possible negative interactions between the Balkan frog and Italian indigenous water frogs (Ficetola and Scali, 2010), no specific study has ever been carried out to explore if and how far *P. kurtmuelleri* is threatening local biodiversity. Hybridization is widespread in water frogs (Holsbeek and Jooris, 2010) and many alien amphibians serve as pathogen vector (Garner et al., 2006; Measey et al., 2016). Carrying out specific studies about these last topics is an urgent need in our opinion, whereby, given the magnitude that this invasion seems to be able to reach, we hope that this manuscript will serve as an incentive for this purpose.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <<http://www-3.unipv.it/webshi/appendix>> manuscript number 20871.

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