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- Doctoral thesis summary -

Usefulness of spatial models in biodiversity management: case studies on amphibians and reptiles

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Publications of the author

With IF:

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Iosif R, Rozylowicz L, Cogălniceanu D. Spatial factors determining inventory bias in distribution atlas of amphibians and reptiles of Romania.

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INTRODUCTION

Amphibians and reptiles play the role of biological indicators, their presence reflecting a healthy ecosystem (Lambert 1997). By studying these organisms the researchers generated important spatial data covering the entire world. The Global Amphibian and Reptile Assessment Working Groups provide updated distribution datasets for all the described species. Other distribution datasets are available at broad scale (e.g., Global Biodiversity Information Facility offered access to 3,745,874 amphibian records and 4,817,569 reptile records at 01.09.2015) or at national level (e.g., Centre de Coordination pour la Protection des Amphibiens et des Reptiles de Suisse is managing the distribution datasets of amphibians and reptiles from Switzerland; AmphibiaWeb Ecuador is managing the distribution datasets of 558 species from this country). For Romania, we recently updated the distribution maps of amphibians and reptiles in Cogălniceanu et al. (2013a, b) but there are no available platforms for data collecting or managing. These examples demonstrate that spatial data are mandatories to evaluate the decline and conservation status of amphibians and reptiles all over the world, but their quality is sometimes under debate.

In Europe there are regions with insufficient data and amphibians and reptiles are organisms with such incomplete distributions (Sillero et al. 2014). In Eastern Europe for example, the lower number of researchers involved in mapping the biodiversity, and sometimes the lack of public participation, are limiting the spatial coverage of the data and generate severe biases between regions. Romania is a good example to conceptualize the factors that generates spatial biases in the distribution datasets:

1. Protected areas are attracting conservation funds and subsequently attract high efforts for biodiversity inventory (Botts et al. 2011). This process describes most of the biodiversity spatial databases (Lobo et al. 2007). Protected areas easily become areas with a complete inventory (i.e., the total number of species living in that area is rapidly inventoried). On the other side, in regions without protection the complete inventory is delayed or never takes place.

2. Places where certain species were historically recorded are attractive. There is a tendency to over sample places where a species was found in the past because it offers the certainty of finding it again. In this way, the number of records became highly clustered in relatively small regions, without contributing to general inventory completeness.

3. Regions with high species richness are attracting conservation funds and the attention of enthusiasts and volunteers. Most of the times, these regions have a complete

inventory but this is not enough to have a proper database since regions with lower richness are literally neglected.

4. Cities with universities or other important research centers provide teams of herpetologists that collect and publish their field data. In this respect, an effect of accessibility appears in terms of the distance towards these cities (i.e., the number of records increase with the decreasing distance towards the cities). Every national attempt to compile distribution data has to take into account this effect of accessibility.

5. Researchers, the general public and volunteers are avoiding the inaccessible regions. In this respect, the collected records became clustered closer to the roads for example.

Spatial modeling implies quantitative assessment of the spatial data and has the advantage that can analyze the biological responses under human disturbances. Most of the disturbances cause a spatial-measureable response. In this respect, spatial models can be used depending on the research question at different spatial scales.

The impact of climate changes can be analyzed at the s**cale of species geographic ranges** (Pearson and Dawnson 2003). In this case the researchers are looking for biological responses such as extinction of endemic species, range losses, range shifts towards higher altitudes and latitudes (Cheung et al. 2009), changes in the overlapping zones of sister species (Iosif et al. 2014), competition disturbances (Costa and Schlupp 2010), or practical conservation hypotheses like overlapping ecological niche with protected areas (Popescu et al. 2013).

Ecological niche modeling is widely used to answer such research questions. This approach correlates species distribution records with environmental variables in order to build the potential ecological niche in environmental space and further to estimate the potential distribution in geographic space (Guisan and Zimmermann 2000). Some of the best known software available are BIOMOD (Thuiller et al. 2009), Maxent (Phillips et al. 2006), and BioMapper (Hirzel et al. 2006).

The impact of habitat losses can be quantified at **landscape level** (Firbank et al. 2008). Researchers are looking for biological responses such as connectivity decrease (Compton et al. 2007), isolation, and population declines (Andren 1994, Moilanen and Hanski 1998). These approaches are useful when both landscape data and distribution/occupancy data are available at long periods of time. Having the two datasets allows testing the species responses to agriculture expansion of the last decades (Burel et al. 2004), to deforestation (Ferraz et al. 2007), etc. Between the most used models I mention graph modeling (Urban and Keitt 2001) implemented for example in Conefor Sensinode software (Saura and Torné 2009), and least cost models (Adriaensen et al. 2003). Furthermore, McRae (2006) transposed the electric circuits' theory into landscape systems in order to estimate dispersal capabilities between populations.

Spatial models can also evaluate **local-scale impacts** such as the impact of man-made microhabitats on a population in terms of abundance decrease (Bender et al. 1998), biased sex ratio (Wolff et al. 1997), etc. In general, models that deal with habitat occupancy and abundance data require repeated visits for long periods of time. For this reason they are mostly used at local scale.

Aim and objectives

The aim of this thesis is to evaluate the usefulness of spatial models in managing the sensitive amphibians and reptiles populations under recent environmental changes.

The thesis consists of four case studies: the first case study evaluates the quality of the distribution data and offers guidelines to identify an incomplete and biased distribution, through both modeling and field testing; the second case study assesses the spatial abundance at local scale to identify the impacts of man-made microhabitats on a population; the third case study approaches the landscape changes at regional scale and evaluates the impact of habitat degradation on landscape connectivity; the fourth case study evaluates the impact of climate changes on species ranges. The connections between these case studies are summarized in Figure 1. The following thesis will provide quantitative support for future conservation strategies of biodiversity in Romania.

The specific objectives for the four case studies are:

1.1. to identify the spatial patterns in the distribution of amphibians and reptiles in Romania; to delineate over- and under-sampled regions.

1.2. to identify the factors determining spatial biases in the sampling effort of the amphibians distribution in Romania.

1.3. to test the spatial patterns previously highlighted by updating the distribution of the Syrian Spadefoot toad *Pelobates syriacus* through extensive field campaigns.

2. to evaluate the impact of archaeological diggings on abundance and viability of a population of the Spur thighed tortoise *Testudo graeca*.

3. to quantify the metapopulation connectivity loss over 36 years for amphibian communities in north-western France.

4. to map the range shifts of two sister species of genus *Pelobates* under climate changes of the past and the future.

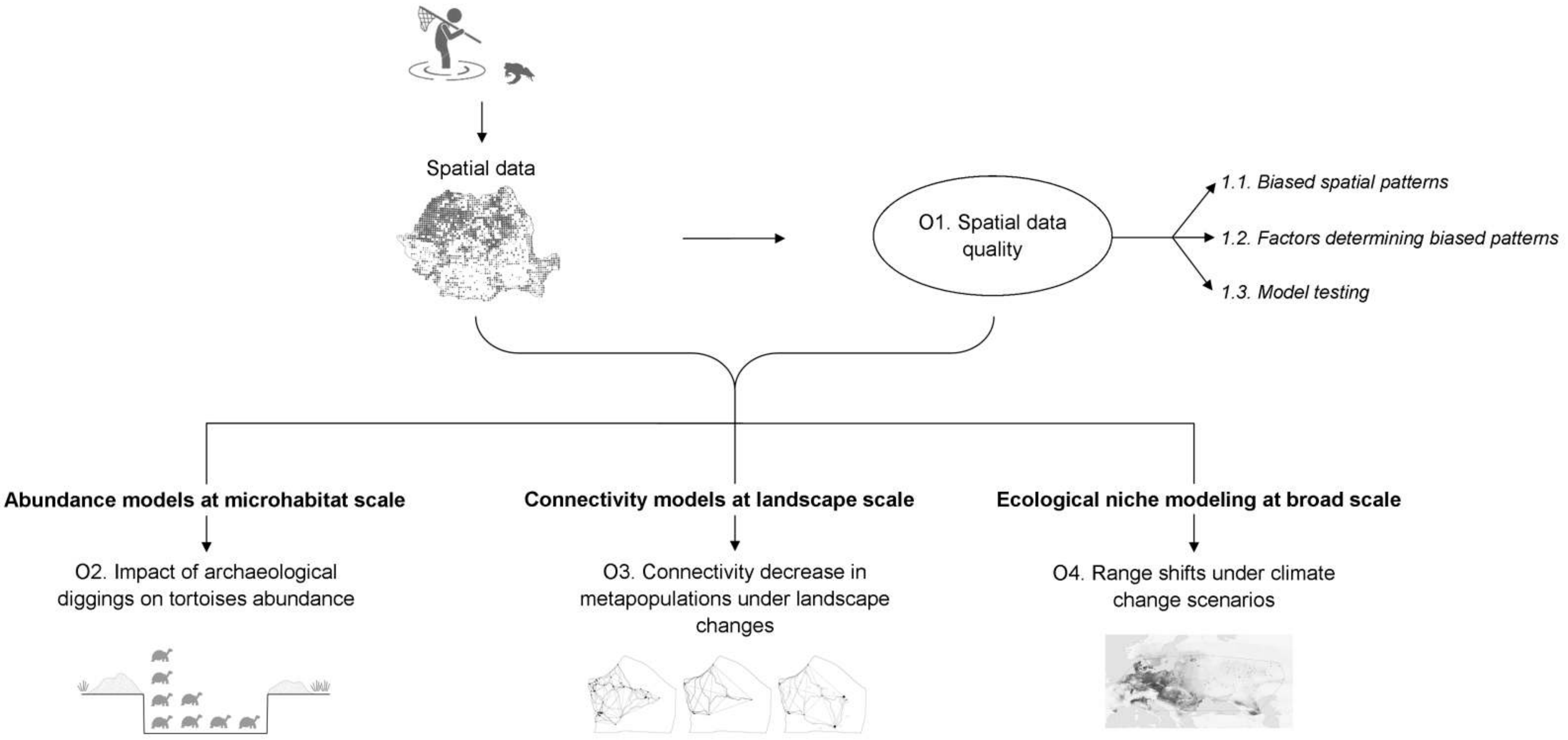


Figure 1 – The relationships between the objectives of the thesis

CHAPTER 1

Spatial bias in the distribution dataset of amphibians and reptiles in Romania

1.1. Distribution of amphibians and reptiles in Romania: spatial patterns, over-sampled regions and under-sampled regions

Introduction

Romania holds healthy aquatic habitats of high conservation value (Rey et al. 2007). For this reason the conservation status of many amphibians and reptiles depends on the viability of the Romanian populations (i.e., *Lissotriton montandoni*, *Triturus dobrogicus*). The reptile diversity is high for a country with mostly continental climate. Twenty-three reptile species live here, 12 of them reaching their range limits in Romania (Gasc et al. 1997). Cogălniceanu et al. (2013a, b) updated the distribution maps of amphibians and reptiles in Romania by compiling all the data cumulated in the past 52 years following the milestone volumes of Romanian Fauna (Fuhn 1960, Fuhn and Vancea 1961). These recent updates allowed assessing the quality of the data. In this chapter I aimed to analyze the spatial patterns of the national distribution data of amphibians and reptiles in order to understand their qualitative limitations.

Methods

Distribution records were aggregated to a UTM 5 × 5 km grid. Then I used Global Moran's *I* test to assess the general trend of spatial autocorrelation in the distribution records. If this test is statistically significant the spatial pattern of the distribution records can be clustered ($Z > 0$), dispersed ($Z < 0$) or random ($Z = 0$) (Fortin and Dale 2005). To delineate the regions where the sampling effort is significantly higher (i.e., over-sampled regions) or lower (i.e., under-sampled regions) than expected by change alone, I used the Getis Ord Gi* statistic (Getis and Ord 2010, Ord and Getis 1995).

To calculate the species richness I increased the resolution of the UTM grid to 50 × 50 km. By using a coarser resolution I removed the local bias in the sampling effort and allowed to better understand regional patterns of species richness (Graham and Hijmans 2006).

Results

For amphibians, the Moran's *I* test revealed a clustered pattern of the number of records per UTM cell (Z = 66.91, $p < 0.001$), suggesting a possible bias in the sampling effort, uneven between regions. For reptiles I obtained a similar clustered pattern but less pronounced (Z = 19.98, $p < 0.001$). Getis Ord Gi* test delineated the regions predicted to be significantly over- or under-sampled for both amphibians and reptiles in Figure 2.

The species richness ranged between 3 and 16 per UTM 50 × 50 km cell for amphibians and between 1 and 17 for reptiles (Figure 3). On the amphibians richness map a constant sampling effort with a complete inventory was revealed for center and western part of the country. On the reptiles richness map an over-sampled pattern was revealed for the agricultural regions of southern Romania.

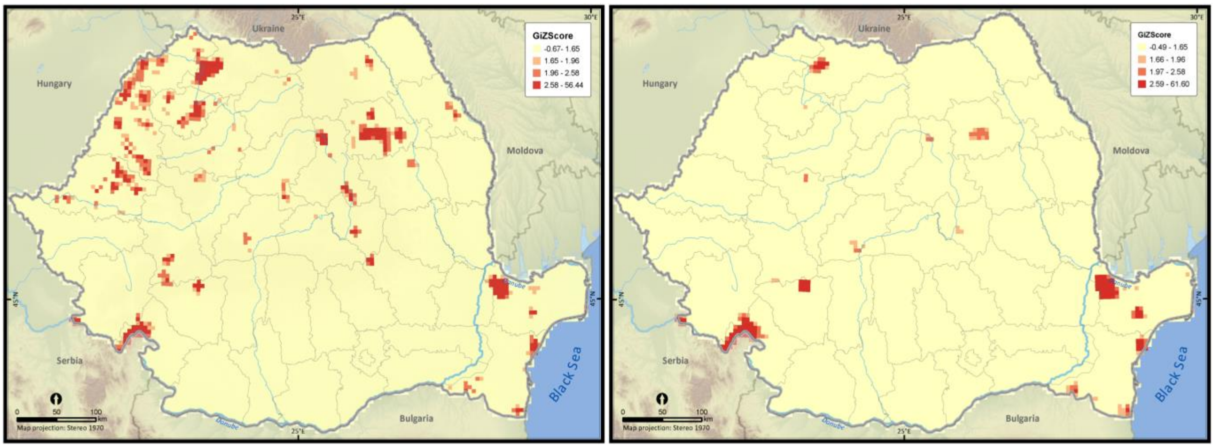


Figure 2 – Over sampled regions in Romania in terms of amphibian (left) and reptile records (right). The *p* value is < 0.05 when the Z scores range between 1.96 and 56.44, for amphibians, and 1.96 and 61.60 for reptiles (shades of red), suggesting a strong clustered pattern of the number of records per UTM 5 × 5 km cell.

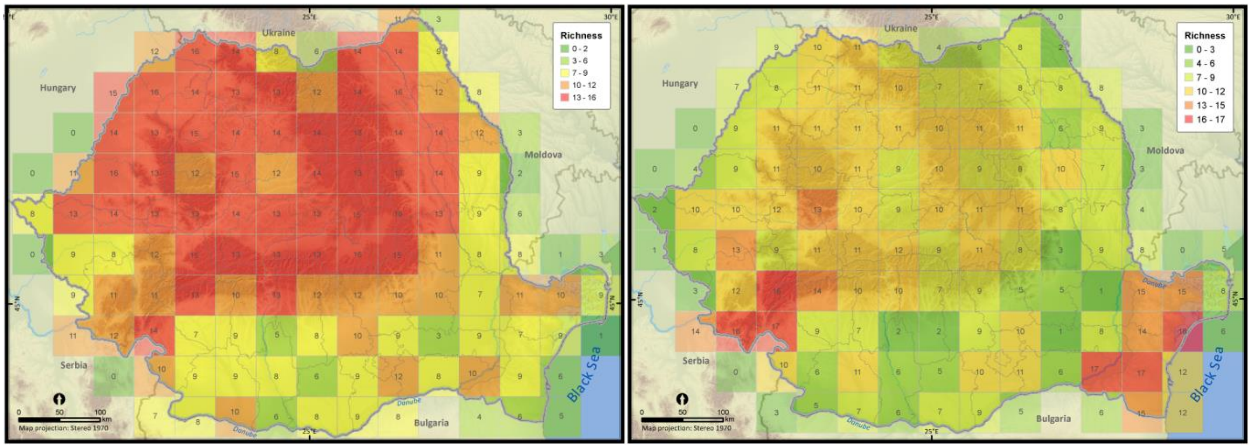


Figure 3 – Amphibian (left) and reptile richness (right) in Romania at a resolution of 50 km.

Discussion

I identified the spatial patterns in the sampling effort of the distribution data and I delineated the over- and under-sampled regions. Over-sampled regions cover mostly protected areas like Jiului Gorges National Park, Măcin Mountain National Park, and Iron Gates Natural Park. This effect of attraction determined by protected areas was reported in other countries and other species as well (e.g., Loureiro et al. 2010, Botts et al. 2011).

Spatial patterns of species richness support the existence of sampling biases especially for amphibians. For amphibians, southern Romania tends to be under-sampled, while an even inventory is made in center and western parts. For reptiles, richness patterns highlight the complete inventory in regions with high richness from south-west and south-east where the climate is warmer and drier (Rodríguez et al. 2005). The rest of the country has more or less a constant inventory. I identified two major under sampled areas that need future inventories for both amphibians and reptiles: Oltenia and Bărăgan Plains. These two regions were probably neglected due to the extensive agricultural landscape there, that determined a lack of interest in research and conservation.

1.2. Factors determining a biased inventory of amphibian distribution

Introduction

The distribution databases have severe biases both spatially and temporally, because most of the distribution data were collected opportunistically (e.g., Botts et al. 2011, Chapman 1999). Bias can have different magnitudes depending on the studied organisms thus limiting the data usefulness, especially when planning conservation at regional and local scales (Hortal et al. 2007). For example, the inventory is almost complete for primates and birds in Equatorial Guinea (high detectability and high attraction) while half of the plants described there have incomplete distributions (low detectability and low accessibility; Zafra-Calvo et al. 2010).

The incomplete and biased inventory of amphibian distribution in Romania was probably caused by factors as: difficult access in regions with low density of roads, complex landscape and topography, lack of funds for large scale and long term inventories. In this chapter I aimed to test the contribution of such factors in determining biases in the sampling effort of the amphibians' distribution in Romania. Specifically, I tested if the inventory was completed or not in relation with altitude, protected areas, and distance towards cities where herpetologists work.

Methods

In order to understand how complete the amphibians' inventory is within the country, I calculated the Inventory Completeness Index for each UTM 50 × 50 km cell. The Inventory Completeness Index is a ratio between the observed richness of a cell and the estimated richness (Romo et al. 2006). The index takes values between 0 (no inventory) and 1 (complete inventory). A complete inventory takes place when the number of estimated species is the same with the number of observed species. To obtain the estimated richness I calculated the rate of finding new amphibian species in a UTM cell using as sampling unit the publications that report amphibian occurrences in that cell (i.e., a rate describing how many new amphibian species are found with every new publication studying a UTM cell). Finally, this estimated species richness was computed using Chao 2 function in EstimateS 9 software (Colwell et al. 2004), resulting the maximum number of species that can be found in each UTM cell (i.e., estimated richness; Colwell et al. 2012).

The Inventory Completeness was then regressed against altitude (extracted from SRTM data, Jarvis et al. 2008, in order to understand to what extent the topographic accessibility influences the inventory), protected areas (I included sites with national protection and Natura 2000 sites in order to understand to what extent the attraction of protected areas influences the inventory), and distance towards cities where herpetologists work (Euclidean distance towards Tulcea, Constanța, Iași, Piatra Neamț, Bucharest, Craiova, Cluj Napoca, Sighișoara, Oradea and Baia Mare, extracted from the publications compiled in Cogălniceanu et al. 2013a (Supplementary file 1 in the full thesis). We refer to these cities as herping cities from now on.

Results

The relation between Inventory Completeness and altitudinal range is positive, Inventory Completeness being higher in mountain areas (i.e., complete inventory) and lower in the lowlands (i.e., incomplete inventory) (Figure 4a). The altitudinal range explains 6.3 % of the inventory variability ($R^2_{\text{Quadratic}} = 0.063$). However, there are regions where altitude better explains this variability (e.g., Western Plain and Bărăganului Plain; Figure 4a).

There is a positive relation between Inventory Completeness and protected areas (Figure 4b). Protected areas explains 4.3% of the inventory variability ($R^2_{\text{Quadratic}} = 0.043$). However, at regional scale the protected areas better explains this variability (e.g., Dobrogea Region; Figure 4b).

The relation between Inventory Completeness Index and distance towards herping cities is negative, the index value decreasing with increasing distance. Distance towards cities explains 3.9 % of the inventory variability ($R^2_{Quadratic} = 0.039$; Figure 4c). Again, at regional scale, there are examples where remoteness or proximity to herping cities has greater influence of inventory (e.g., southern part of Banat region and central part of Moldova region, Figure 4c).

Discussion

In this chapter I evaluated the relative influence of attraction and accessibility factors on sampling effort of amphibians in Romania. I tested the relation between sampling effort (expressed as Inventory Completeness) with altitude, protected areas, and distance towards herping cities.

The relation between inventory completeness and altitude suggests that researchers intensively sampled the mountains rather than lowlands, perhaps considering them more attractive. For example, 27% of the publications in the annexes of Cogălniceanu et al. (2013a) are focused on the mountains but if we remove the high attraction of the Danube Delta and Dobrogea Region the bias will be even higher. The relation between inventory completeness and protected areas suggest that protected areas influence the sampling effort at national level. This has implications in amphibians' management because large areas are under-sampled (e.g., Danube Floodplains, lower course of Olt river, Bărăganului and Siretului Inferior Plains, where many aquatic habitats are present within the agricultural crops). The relation between Inventory Completeness and distance towards herping cities has a certain limiting effect on usefulness of distribution databases. Such biases generate over and under-sampled regions.

The national databases compiled from multiple sources over long periods of time may create a false image to the international policy makers regarding the species distribution and abundance patterns. Future conservation strategies have to take into account these biases in the data.

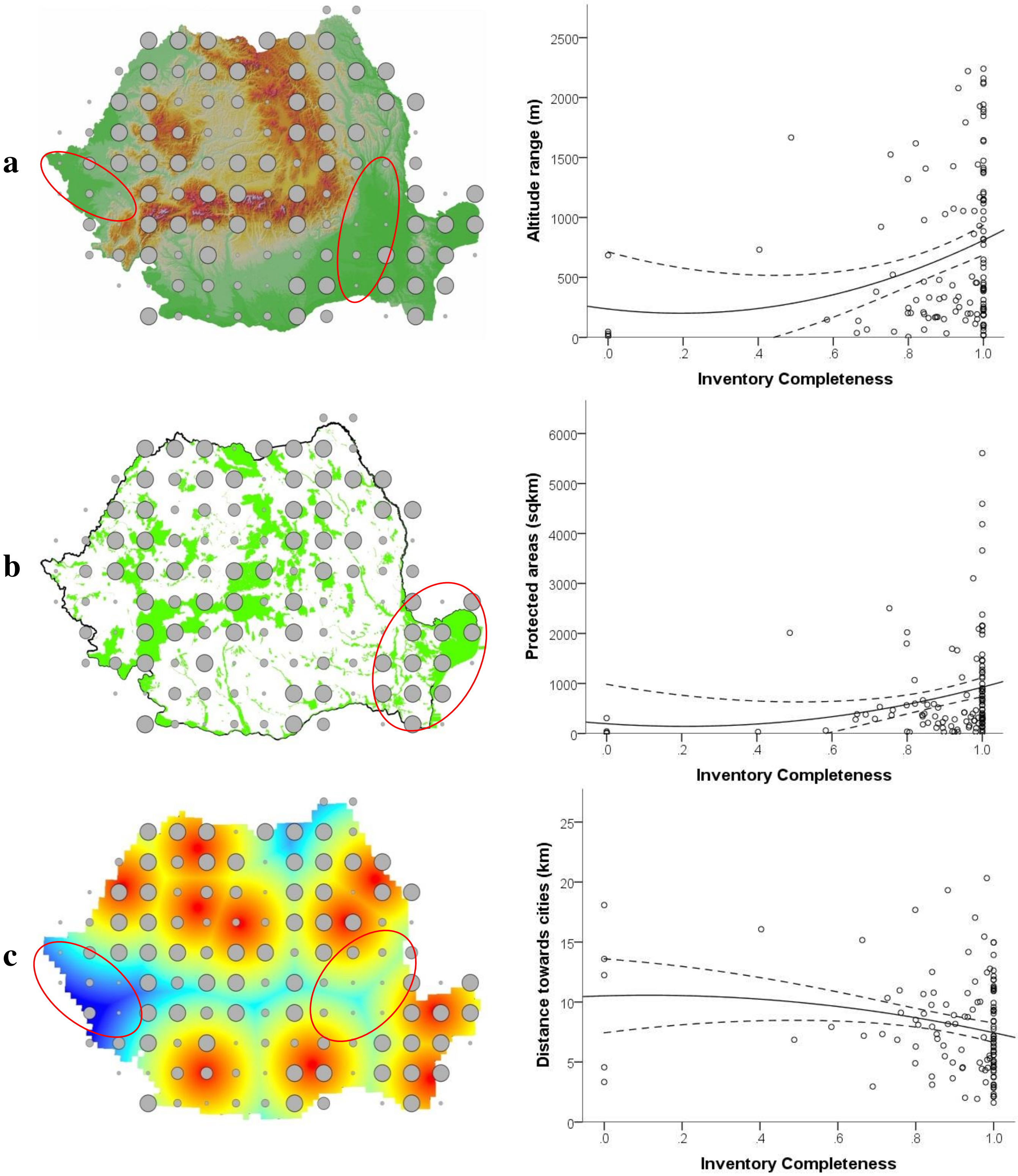


Figure 4 – Inventory Completeness regressed against the factors that explain sampling biases in amphibians' distribution. In panel (a) I plotted Inventory Completeness (size of the grey symbols increases from 0 to 1) against altitude, in panel (b) I plotted Inventory Completeness against protected areas, in panel (c) I plotted Inventory Completeness against distance towards herping cities. On the maps I highlighted in red ellipses the regional trends described in the Results section. The general trends are highlighted on the scatter plots. The black line represents the quadratic function used to describe these trends. The dotted lines represent the 95% confidence interval.

1.3. Testing the spatial patterns: range extension of the Syrian Spadefoot toad *Pelobates syriacus*

Introduction

Two of the four species of Spadefoot toads genus *Pelobates* occur in Romania and both of them reach here their range limits: southern limit for *P. fuscus* and northern limit for *P. syriacus*. These toads have a narrow ecological niche: their habitat structure strongly influence the dynamic and abundance of the populations. They need loose and sandy soil to dig burrows (Nöllert 1990). *P. syriacus* is distributed in south-eastern Balkans, Caucasus, northern Iran, Turkey and parts of the Middle East towards Israel (Džukić et al. 2008, IUCN, 2013). The presence of this species is difficult to confirm because it is strictly nocturnal, has low underwater breeding call, and has cryptic coloration. In Romania, it was described for the first time in 1954 (Băcescu 1954). Moreover, the tadpoles of this species cannot be differentiated from those of its sister species *P. fuscus* (Sidorovska et al. 2002), making almost impossible to determine its presence based on tadpoles only. In this chapter I aimed to evaluate the inventory completeness of *P. syriacus* in Romania (as published in Cogălniceanu et al. 2013a) through extensive field work.

Methods

I collected new distribution records during an extensive field campaign in April-July 2013. During the night I sampled both terrestrial and aquatic habitats and searched for adults and metamorphs. During the day I sampled aquatic habitats only, and collected tadpoles from every pond where metamorphs or adults were not found at night. The tadpoles were brought to the laboratory and kept until species was determined with certainty. Finally they were released in the same sites. The road-killed individuals were also successfully used to document the species presence.

Results and discussion

In this chapter I documented the presence of *P. syriacus* in 36 new localities in Romania. In Galați County I extended the known distribution with 40 km northwards. Moreover, *P. syriacus* was abundant in Bărăgan Plain (Brăila and Buzău Counties). Regarding the western limit of its distribution in Romania, the species was found 50 km westwards from the last locality. Along the Danube (Olt and Giurgiu Counties) the distribution was found to be more compact than known before.

The distribution map in Cogălniceanu et al. (2013a) presents a fragmented distribution of *P. syriacus* in the agricultural landscape of southern Romania. In this chapter I found the species to be rather common and abundant there. The species adapted well and tends to take advantage of the irrigation system that creates a plethora of aquatic habitats. They reproduce in the irrigation canals and the nearby ponds and reservoirs. They feed in the arable crops and pastures and bury in the loose plowed soil. Examples of such viable populations in similar habitats are described in Bonk and Pabijan (2010) for the sister species *P. fuscus*.

The findings in this chapter have implications on conservation status of the species in Romania and European Union. I support the idea of a sampling bias in the national maps of amphibian distribution, not only for this species but probably for other species as well. I demonstrated that under-sampled regions and species exist (i.e., Danube floodplains, Bărăgan Plain for *P. syriacus*). I suspect that the main reason to which these regions are under-sampled is the lack of attraction for herpetologists due to the large agriculture extension.

CHAPTER 2

Advantages of archaeological sites: positive impact of archaeological diggings on Spur thighted tortoise

Introduction

Biodiversity conservation creates and manages protected areas where it limits human disturbances, thus contributing to a better conservation status for habitats and species. In practice, conservation strategies have neglected the importance of lands with restricted access due to other reasons than nature protection. These lands have the potential to strengthen the network of biodiversity protected areas since most of them shelter a high number of species and viable populations. Sites of cultural heritage like archaeological sites are a good example, still poorly explored. Human disturbances are usually limited in archaeological sites, only research and touristic activities being allowed. Recently, Vanderplank et al. (2014) recommended that archaeologists and conservationists should develop shared protection strategies of floristic diversity within archaeological sites of Baja California. But examples like this are rare; the real importance of such situations is neglected.

Case study: Spur thighted tortoise at Histria Archaeological Complex

Archeological research started at Histria in 1914 and intensified in 1950-1970 (Vulpe 2014). The archaeologists left behind diggings with depth ranging from 0.5 to 4 m and surface ranging from approximately 10 m^2 to 150 m^2. After completing the intensive digging, 10 ha of ruins were opened for tourists and 32 ha were enclosed with restricted human access (I refer to this as closed archaeological site from now on). The closed archaeological site became home to a population of the vulnerable species *Testudo graeca*. The population was studied since 2010 and the results suggest is a young population, with favorable health and conservation statuses, and long term viability perspectives (Buică 2013, Buică et al. 2013). The population is isolated within the closed archaeological site where landscape is favorable Pontic steppe, while the surroundings are dominated by wetlands and lakes. The population size in 2012 was estimated at 221 ± 12.2 individuals and the observed density was 5.1 individuals per hectare (Buică et al. 2013).

Within the close archaeological site the tortoises seems to take advantage on the closed and eroded diggings. The diggings diversify the microhabitats of the homogenous grasslands. They bring higher and better drained slopes that are probably safer as hibernation and nesting sites. The diggings' walls have bare and loose soil, probably allowing the tortoises to dig easier and deeper. All these aspects can be important for a population living at the northern edge of the species distribution where low winter temperatures are a major threat.

During my field work within the closed archaeological site I observed hibernaculas clustered and stacked on the slopes of the archaeological diggings. Moreover, I observed tortoises active or basking inside the diggings at the start of the season in March and towards the end of the season, right before the hibernation. At the begging of the summer I observed females searching for safe places to lay their eggs or predated nests, inside or close to the diggings. These observations were conceptualized in Figure 5.

In this chapter I tested the hypothesis that seasonal abundance of tortoises is spatially dependent by the microhabitats created by the archaeologists. I aimed to answer the following questions: (i) is the tortoises' abundance higher inside archaeological diggings right after and right before hibernation? and (ii) is females abundance higher inside the archaeological diggings during eggs laying season? Finally, I discussed the issue of the population viability in the framework of archaeological and biodiversity conservation.

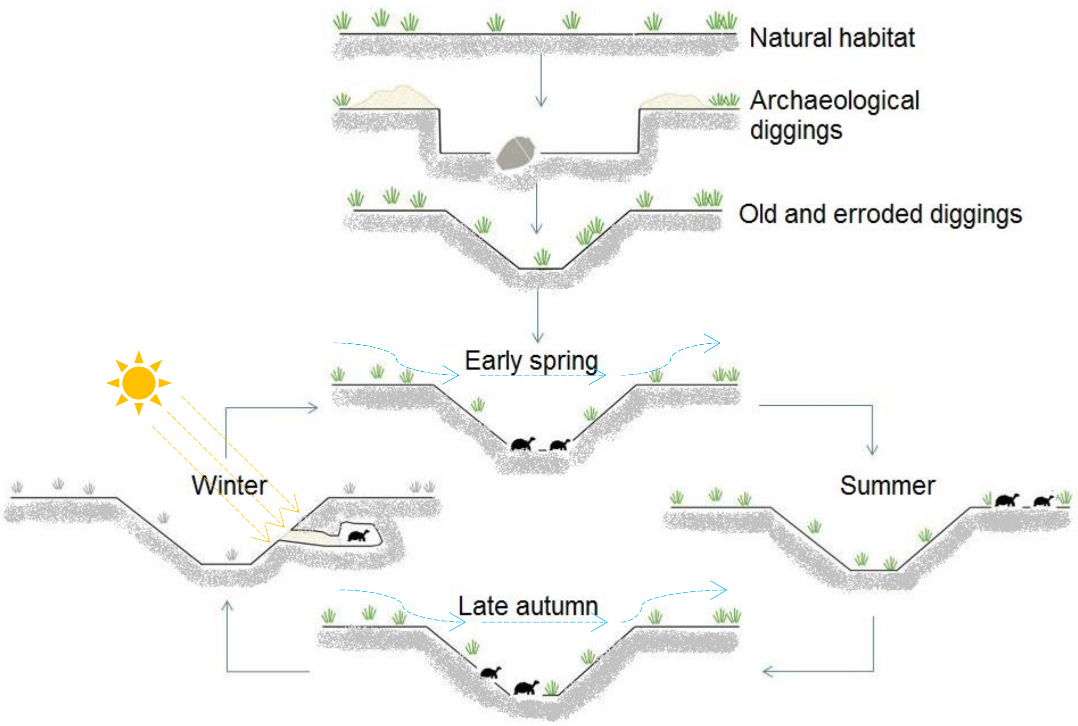


Figure 5 – The ecological processes observed in the Spur thighted tortoise in the closed archaeological site. After the archaeological research stopped the diggings eroded and became part of the tortoises' habitat. Moreover, the tortoises tend to take advantage on the new microhabitats that offers safe places for nesting and hibernation, and shelter from the cold weather in spring and autumn.

Methods

Between 2010 and 2015 we searched for tortoises within the perimeter of the closed archaeological site using random transects. Buică (2013) collected the field data between 2010 and 2012 while I and a team of students collected between 2013 and 2015. First period had a low sampling effort with only one researcher going in the field per day. Second period had a higher sampling effort; in average ± Std. Dev, 2.2 ± 1.3 researchers per day sampled the site. Besides live tortoises, we marked the GPS location of death individuals, predated nests, and hibernaculas, every time we were certain they belong to *Testudo graeca*. Every new individual was permanently marked.

To calculate the tortoises' abundance I created a grid of 10 m resolution covering the entire area of the closed archaeological site. I counted the number of sightings in each grid cell for every month but independent of the year. Spatial patterns of abundance were then analyzed using Getis-Ord Gi* (Ord and Getis 1995, Getis and Ord 2010) and Getis-Ord General G (Ord and Getis 1995) statistics. These two statistics were calculated on the 10 m grid for every month and separately for males, females, and all individuals in the same scenario. Getis-Ord Gi* statistic allows to delineate hotspots of abundance while Getis-Ord

General G describes the general pattern of abundance in space (i.e., if the spatial pattern is clustered or random).

The archaeological diggings were digitized from aerial images at a resolution of 0.5 m. I finally overlapped the results of Getis-Ord tests with the diggings in order to identify the months where the clustered pattern is due to them.

Results and discussion

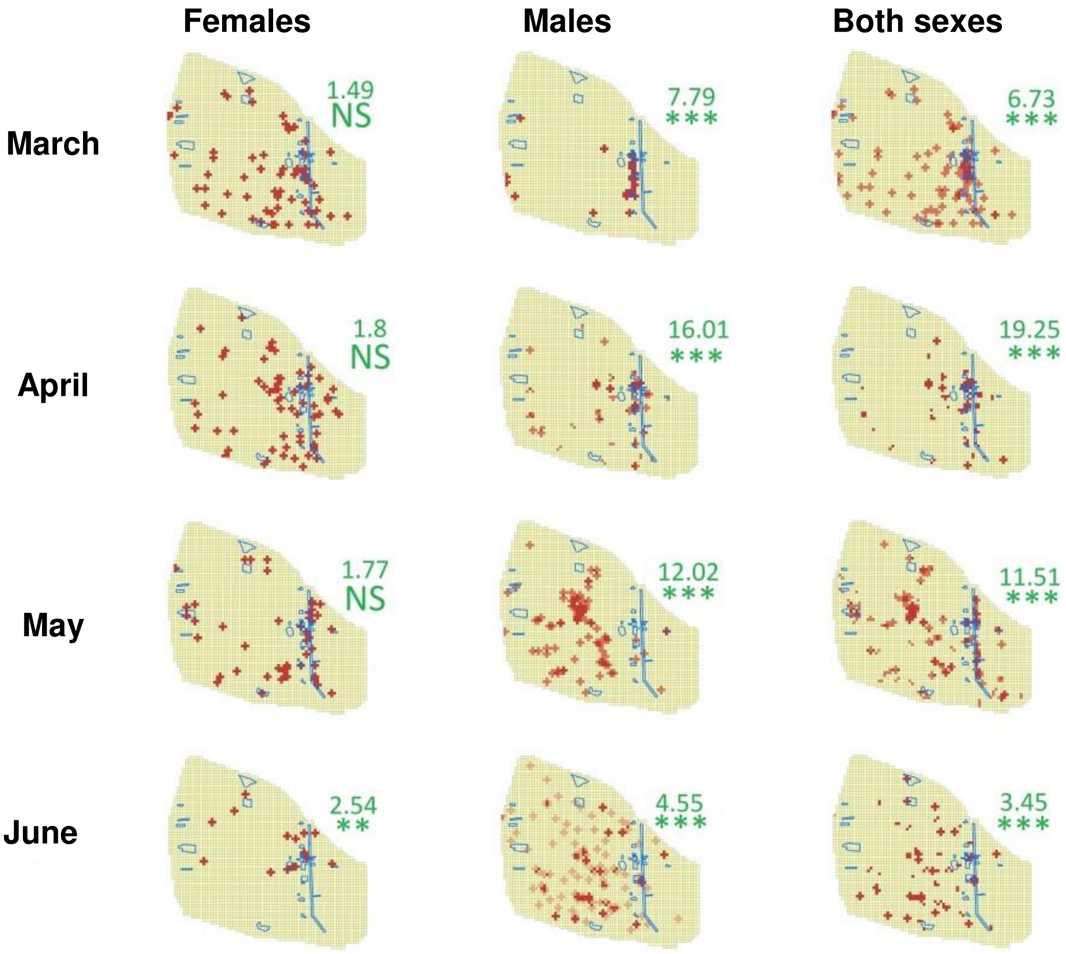
The total number of tortoises marked at the archaeological complex increased from 170 in 2012 to 385 in 2015 (includes both sexes and all developmental stages). The observed density increased from 5.1 to 12.1 per hectare due to the higher sampling effort. The density is higher than in populations from Spain (Andreu et al. 2000, Ballestar et al. 2004), Greece (Hailey 2000), and Morocco (Slimani et al. 2002, Kaddour et al. 2006). Adults' weight was slightly higher for the second study period ($\text{Mean}_{2010\text{-}2012} = 1768 \pm 411$g, $\text{Mean}_{2013\text{-}2015} = 1976 \pm 527$ g; $t(165) = -3.24$, $p = 0.001$). Adults represent 67.7% of the population, of which females have 35.5 % of the population and males 32.2% (sex ratio = 1.1), subadults 12.7 %, while juveniles 19.4 %. The weight of the adults and the population structure indicates that population reproduces successfully, is young and viable in the habitat modified by the archaeologists.

The spatial patterns highlighted a random distributed abundance for females between March and May (Figure 6). This result suggests the females dispersed within the archaeological site after they exit hibernation. In June and July, the female abundance is clustered within the archaeological diggings suggesting their need to find a proper nesting site. In August and September the abundance is randomly distributed all over the archaeological site suggesting the females are looking for food before the cold season. In October the females have a lower detectability and a lower abundance clustered inside the archaeological diggings (Figure 6). For males, the abundance has a strong clustered pattern in March inside archaeological diggings (Figure 6). The same pattern was observed in April, with some exceptions of individuals starting to disperse outside the archaeological diggings. In May-June, there is another clustering pattern for males but this time the abundance is concentrated in the central part of the site, where I observed plant communities with *Vicia craca*, an important food resource for the tortoises in this region. In July-August, the males' abundance is clustered at the diggings system where they were observed digging aestivation holes. In autumn, males retreated inside archaeological diggings where they hibernate alongside females (Figure 6). Still, telemetry studies spanning over 10-20 years found that habitat/microhabitat selection differ among individuals of the same population (Seibert and

Belzer 2013). We suggest caution is needed to conclude that all individuals of Histria population equally take advantage on archaeological diggings.

Conclusions

This chapter proved that tortoises' abundance depends along the season on the archaeological diggings. Nevertheless, diggings modify their habitat, finally putting them in advantage against environmental constraints. Tortoises take advantage on the diggings to shelter against extreme temperatures, to dig nests and hibernaculas. Archaeological sites have a high level of protection while the surroundings have low biodiversity due to human disturbances. An aspect need to be further capitalized: integrative biodiversity and cultural heritage conservation.



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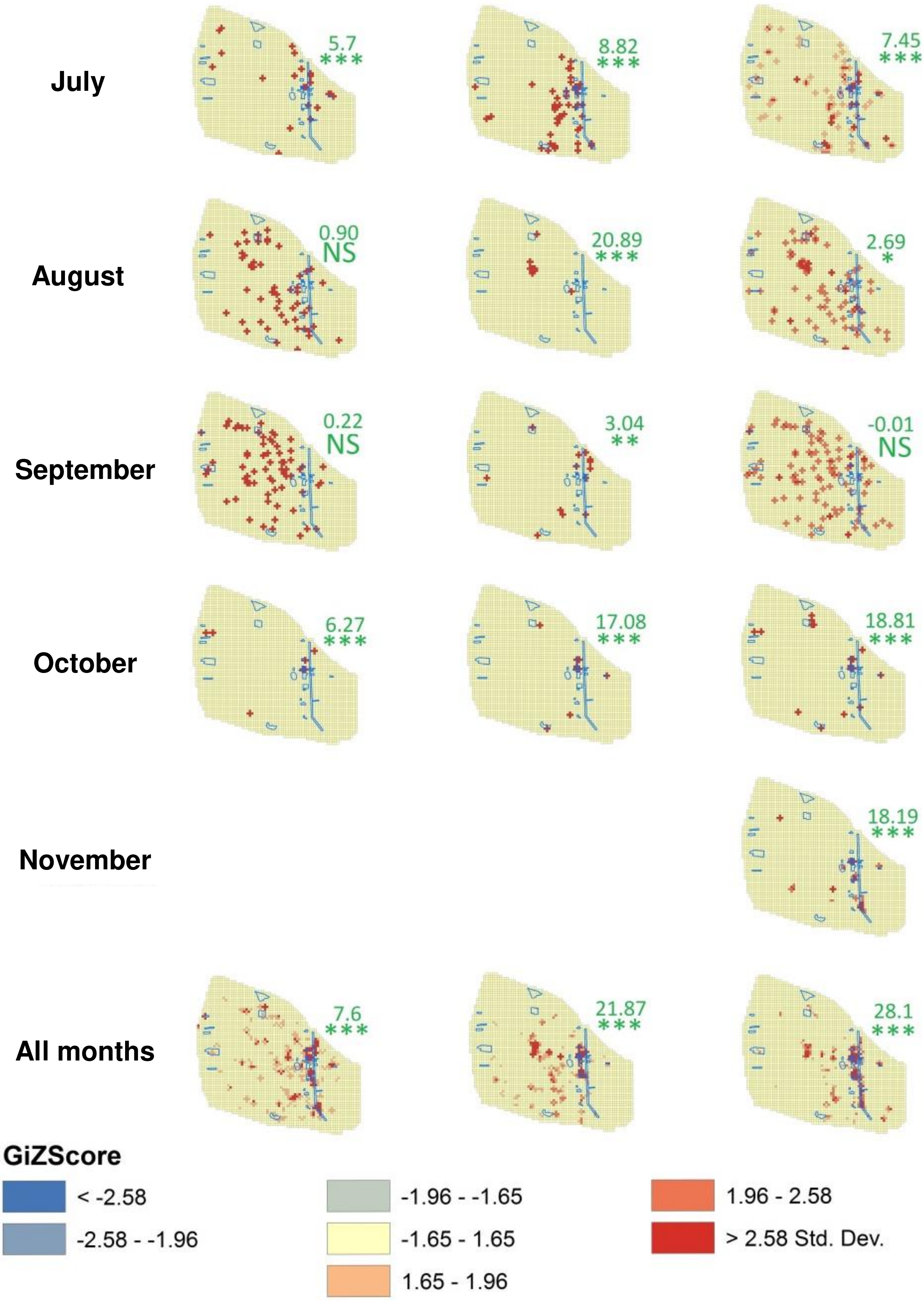


Figure 6 – Abundance spatial patterns of tortoises within the closed archaeological site of Histria Archaeological Complex. The abundance is plotted as number of individuals per 10×10 m cell and then evaluated with Getis-Ord Gi* spatial statistic. Z scores returned by this test are plotted in yellow to red shades. Z scores returned by Getis-Ord General G statistic are written in green. For this last statistic NS refers to non significance, * – *p* between 0.05 and 0.01, ** – *p* between 0.01 and 0.001, *** – *p* between 0.001 and 0. Archaeological diggings are mapped with blue polygons.

CHAPTER 3

Metapopulation connectivity of amphibians in north-western France

Introduction

Most of the organisms are distributed in metapopulation structures, their viability being dependent on their dispersal capabilities (Beier et al. 2008). In practice, nature conservation frequently assumes protection and restoration of wildlife corridors and other habitats that favors dispersion (Sutcliff et al. 2003, McRae et al. 2012).

Many Holarctic amphibians have a metapopulation structure. Most of them deposit large number of eggs or larvae in stagnant waters. After larval development and metamorphosis, part of the juveniles disperse, and their success will depend on both ponds availability and quality of the terrestrial habitats they have to cross (Marsh and Trenham 2001, Mazerolle and Desrochers 2005). In this chapter I evaluated the amphibian populations' connectivity as a function of aquatic and terrestrial habitat loss over 36 years in a rural area of north-western France. Finally, I evaluated how the creation of new ponds restored the populations' connectivity.

Methods

I studied the western, coastal part of Pas de Calais department in north-western France. The landscape is agricultural, being dominated by crop lands and pastures.

Amphibians' surveys were performed by Arntzen et al. in 1974-'75 (first survey, I - 208 ponds) and in 2011-'12 (second survey, IIa - 166 ponds). Before 2012 the administration of the Natural Regional Park Caps et Marais d'Opale created 22 new ponds. Thus, a third survey (i.e., IIb) took place in 2012 and sampled only these new ponds. In these surveys, Arntzen et al. found 12 species of amphibians that can be classified according to their abundance: i. common species - *Lissotriton helveticus*, *L. vulgaris*, *Ichthyosaura alpestris*, *Bufo bufo* and *Rana temporaria*; ii. species of moderate occurrence not in decline - *Epidalea calamita* and *Hyla arborea*; iii. species of moderate occurrence in decline - *Triturus cristatus* and *Alytes obstetricans* and iv. rare species - *Salamandra salamandra*, *Pelodytes punctatus* and *Pelophylax kl. esculentus*.

Land use data are available for 1963, roughly corresponding to the first survey, and for 2003, roughly corresponding to the second survey (see Curado et al. 2011). Impediment to dispersal was estimated by assigning resistance values to landscape classes on the basis of the habitat preference of the species, through expert opinion (see Zeller et al. 2012 for a general discussion). Values of the resulted resistance layer ranged from zero for no impediment to dispersal to unity for a complete barrier to dispersal. The resistance values applied are 0.0 for ponds, 0.1 for marshes and un-vegetated dunes, 0.2 for vegetated dunes and quarries, 0.3 for pasture, 0.5 for forest, 0.9 for arable and transport and 1.0 for urban.

I represented the dispersal routes from the ponds of origin until reaching a new pond for reproduction using: i. Euclidian distance links between ponds with the species reported present, and ii. least cost corridors based on the resistance layer, both generated in Linkage Mapper software (McRae and Kavanagh 2011). Links were used to derive two landscape resistance indices for amphibian dispersal: total resistance which depends on ponds density and relative resistance which depend on the resistance to dispersal of terrestrial habitats. Temporal changes in links and corridors were tested for statistical significance using Mann-Whitney U test.

Results

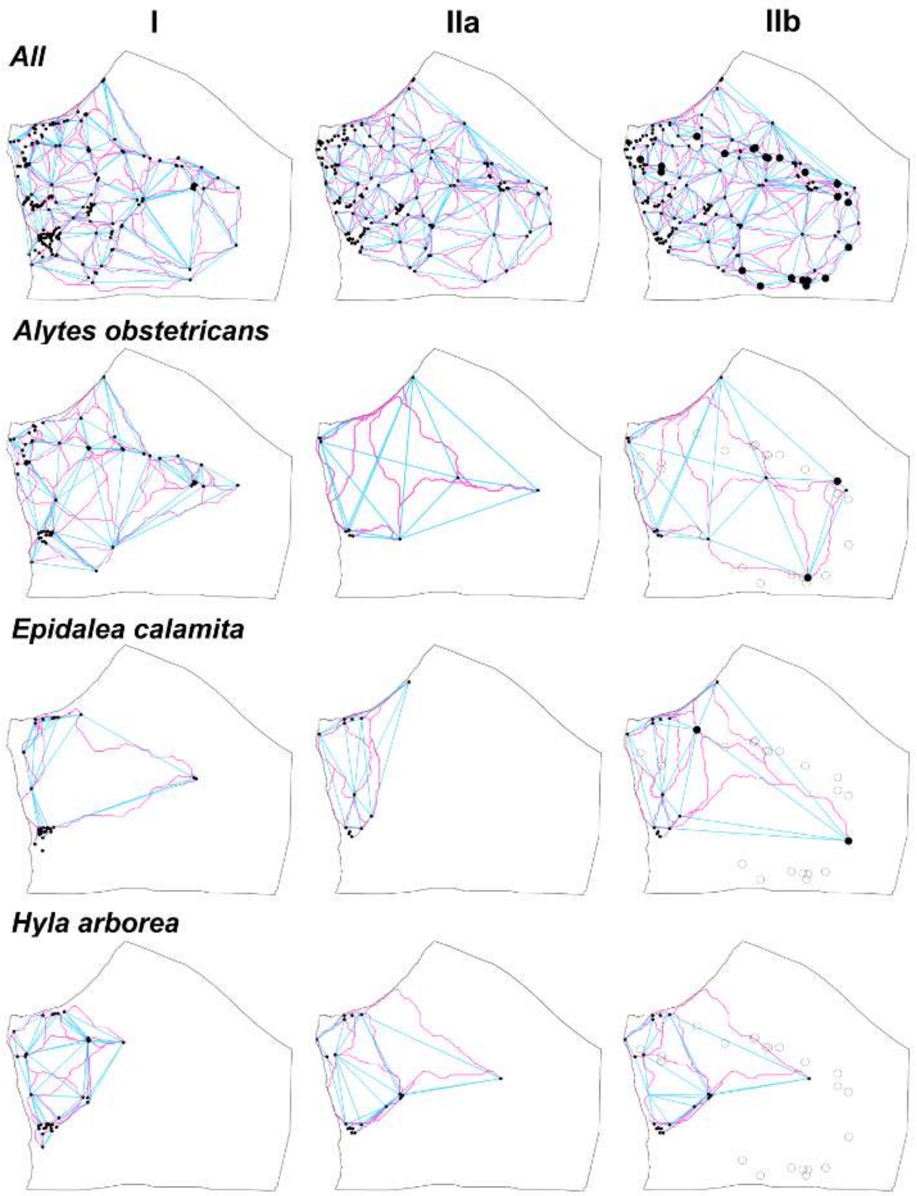
The number of ponds with amphibians is 208 in the first survey (I) and 166 in the second survey (IIa) with a corresponding increase in the average length of links (Figure 7). Calculated as corridors, dispersal routes increased significantly for *A. obstetricans* ($p < 0.001$) and for *T. cristatus* ($p < 0.01$) but not for the other species or the group as a whole. The creation of new ponds had no marked effect on these results (Figure 7).

The landscape total resistance against dispersal increases between surveys I and IIa from 1.89 ± 1.21 la 4.71 ± 2.53 for *A. obstetricans* ($p < 0.001$) and from 2.01 ± 1.12 to 4.95 ± 3.14 for *T. cristatus* ($p < 0,05$). For the other scenarios (the group as a whole, *E. calamita*, and *H. arborea*, the difference between the two surveys is not significant ($p > 0.05$)). Relative resistance increases from 0.58 ± 0.14 to 0.60 ± 0.17 for the group as a whole ($p < 0.01$) but not for individual species.

Discussion

I aimed to understand if amphibian populations have a lower connectivity after 36 years of landscape changes in Pas-de-Calais department, north-western France. The main results of this chapter showed how connectivity decreased between the two surveys and that the decline is explained by both pond losses and terrestrial habitat decline in terms of resistance to dispersal. These two factors have different implications from species to species. When all the species were considered as a group, the results showed a decrease in the connectivity due to the changes in the terrestrial habitats (i.e., arable expansion over pastures) and less due to the pond losses. These changes reduce the dispersal capabilities of amphibians, weakening the metapopulation processes like gene flows (Spear and Storfer 2010, Trumbo et al. 2013). For *T. cristatus* and *A. obstetricans* the results showed the individuals have to cross longer distances during migrations, because suitable ponds almost disappeared for these species.

Creating new ponds is effective, more than restoring the terrestrial habitats for species like *T. cristatus* and *A. obstetricans*. Still, limiting the arable expansion is expected to favor all amphibian species as demonstrated in other parts of Europe (Hartel et al. 2010).



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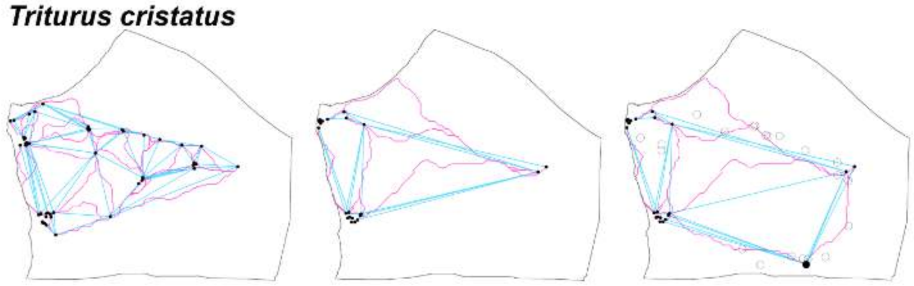


Figure 7 – Links (Euclidian distance lines in blue) and corridors (purple lines) for amphibian species studied in Pas-de-Calais department of north-western France. Ponds where species were reported present were plotted as black dots. I refer to the first survey, IIa refers to the second survey, and IIb refer to the last survey that focused on the 22 newly created ponds. On the maps of this last survey the closed black circles represent the new and occupied ponds and the open black circles represent the new and unoccupied ones.

CHAPTER 4

Ecological niche and range shifts of the spadefoot toads genus *Pelobates* under climate change

Introduction

Climate change contributed to the decline and even extinction of many amphibian species throughout the world (Reading 2007). Biological responses to climate change often result in range shifts (Parmesan 2006), usually towards higher altitudes and latitudes, in accordance with the species' thermal limits. For species to survive, they must keep pace with the climate shifts and adapt (Loarie et al. 2009).

The four species of the spadefoot toad genus *Pelobates* (i.e., *P. cultripes*, *P. varaldii*, *P. fuscus*, and *P. syriacus*), distributed over parts of Europe, North Africa, Caucasus, and the Middle East, have mostly allopatric ranges, except for *P. fuscus* and *P. syriacus*. The ranges of the latter two species overlap along a narrow contact zone in Dagestan and in the Balkans, along the Danube between the Black Sea Coast and the Iron Gates. Thus, *P. fuscus* and *P. syriacus* can be used as a case study to understand the factors that shaped the present distribution of closely related amphibians.

In this chapter, I aimed to identify the potential changes in the two species' distributions and particularly the spatial shifts of the overlapping zone. My goals were to (i) compare the two species' niche similarity over different time spans and scenarios, (ii) identify past climatic refugia, and (iii) identify the potential shifts induced by future climate changes in the species' distributions.

Methods

I used a database of species occurrences (4.972 for *P. fuscus* and 394 for *P. syriacus*) compiled from published papers, natural history museum specimens, information available in online databases (www.gbif.org and http://nhm-wien.ac.at/), and from our own field surveys (see Supplementary file 2 in the full thesis), covering the entire extent of the species' ranges. To validate the past projections of the niche models I used fossil records dating in the Last Interglacial - LIG extracted from Lisanfos KMS database (Martín and Sanchiz 2013), FOSFARBASE (Böhme and Ilg 2003), and Holman (1998).

I extracted seven variables from WorldClim climatic data (Hijmans et al. 2005): annual mean temperature, isothermality, temperature seasonality, minimum temperature of coldest month, precipitation seasonality, precipitation of warmest quarter, and precipitation of coldest quarter. For past climatic condition I used two simulations for Last Glacial Maximum - LGM (i.e., MIROC and CCSM; Collins et al. 2006), and one scenario for LIG (Otto-Bliesner et al. 2008). To evaluate the range shifts expected under future climatic conditions I used the A1B scenario derived by IPCC under program The Third Hadley Global Circulation Model. I used this scenario for three time spans: 2030, 2050 and 2080.

I used the Maxent algorithm proposed by Phillips et al. (2006) to generate models of the ecological niche for the two spadefoot toad species and to project the models onto both past and future climate scenarios. To compare the interpredictibility between present and fossil records I calculated the Omission Error as follows: I trained a model based on present records then I projected it into the past and calculated Omission Error using fossil records, and vice versa.

I used the raw Maxent outputs to calculate Schoener's D index, a similarity index of niche space derived by Schoener (1968) and implemented in ENMTools software (Warren et al. 2008). This index ranges between 0 (no similarity between niche models of a species pair) and 1 (identical niche).

Results

Potential ecological niche derived in the climatic space extended outside the known range in the Dagestan Region, Georgia, and Azerbaijan for *P.fuscus*, and westwards in the Mediterranean (e.g., Italian Peninsula) and northwards in the Pannonian Basin and Caucasus for *P. syriacus* (Figure 8a).

The LIG suitable region included the British Isles, France, as well as the vast areas in Northern Europe (e.g., Jutland Peninsula; Figure 8b) for *P. fuscus*, and extended northwards in the Balkans and Caucasus for *P. syriacus.* I obtained a good predictive performance of the fossil records when I projected the niche model of present climate towards LIG climate for both *P. fuscus* (Omission Error = 4.1 %) and *P. syriacus* (Omission Error = 16.6 %). The LGM climatically suitable region was restricted to Pannonian Basin, Italian Peninsula, Carpathians and Caucasus for *P. fuscus*, and to only three main refugia for *P. syriacus*: Israel, Caucasus, and Southern Balkans (Figures 8c, d).

Models projected on climate conditions of the end of the XXI century indicated that the western part of *P. fuscus* range would preserve the climatic suitability, whereas a decrease would occur in the central part (Figure 8e). Climatic suitability of *P. syriacus* tends to decrease in the southern part of its range (25–30° N) (Figure 8e). Higher latitudes will have a higher climatic suitability for both species (Figure 8e).

Niche models from present and LIG have similar values of Schoener's D similarity index ($D_{PRESENT} = 0.30$, $D_{LIG} = 0.26$) while values calculated for LGM are lower ($D_{CCSM} = 0.15$, $D_{MIROC} = 0.20$). Niche similarity was forecasted to rapidly rise until 2080 ($D_{2080} = 0.60$).

Discussion

The ecological niche models for the present climate predicted potential distributions that extended outside of the species known ranges, especially southwards for *P. fuscus* and northwards for *P. syriacus*. These results suggest that their geographic distributions are not at equilibrium with climate. I suggest other factors are responsible for limiting their range overlap, like competition and the presence of geographic barriers.

The climate during the LIG period delineated a distribution that extended westwards for *P. fuscus* and northwards for *P. syriacus*, leading to a higher niche similarity and potential range overlap. The northward extension of the climatic suitability of *P. syriacus* under LIG scenario covered the Pannonian plains, Ukraine, and Dagestan Region. Probably, the Carpathian and Caucasus Mountains acted as geographic barriers as no fossil records were found there. My models are consistent with phylogenetic studies revealing that mountain barriers limited the distribution of other European amphibians (Arntzen et al. 2007).

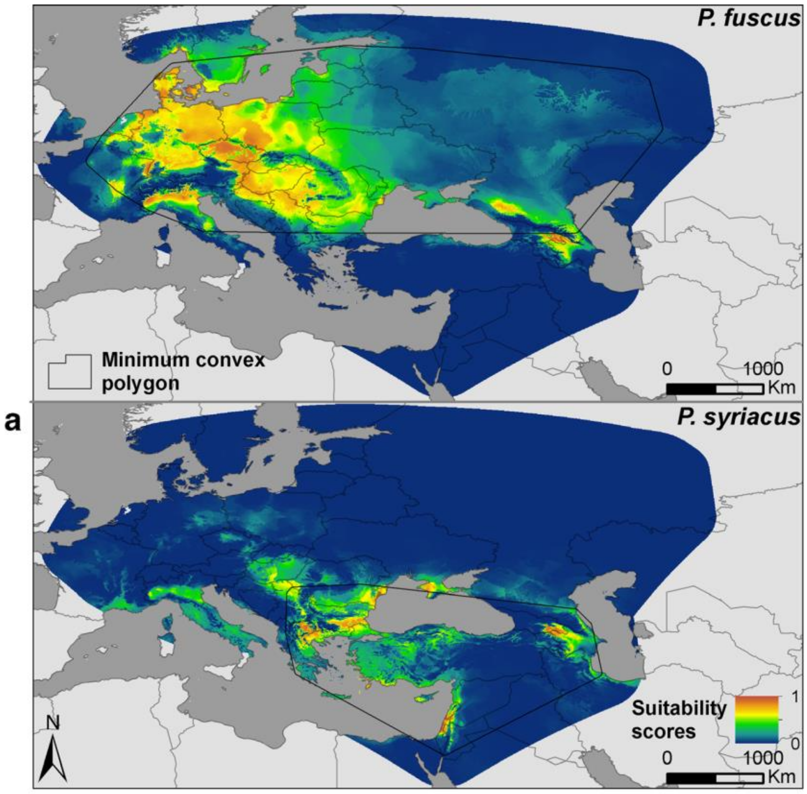
The climate of LGM shaped refugia for both species roughly in the same regions as the overlapping zone of the present day models (i.e., the lower Danube and the northern Caucasus), in agreement with refugia predicted for other amphibians (e.g., genus *Triturus*; Wielstra et al. 2013).

Under future climatic conditions, niche similarity showed a rapid increase. For *P. fuscus*, the western part of the range was projected to maintain climatic suitability, the center part to lose it, and the eastern part to increase it. For *P. syriacus*, models predicted that populations occupying the southern limit will face range loss while those occupying the northern edge will tend to expand further north. These predictions are in accordance with Popescu et al. (2013), who evaluated the potential range shifts of Romanian amphibians and reptiles.

Even where the climatic space will stay favorable, the severe decline due to human disturbances will threaten these spadefoot toad species (Nyström et al. 2002, Bonk and Pabijan 2010).

Conclusions

The present ecological niche models showed a distribution that is not at equilibrium with the climate for both species. Towards the end of the XXI Century, the northern territories will provide the two species a better climatic space. Ecological niche similarity was projected to increase in less than 100 years, suggesting that these rapid climate changes have the potential to disturb the links between the abiotic and biotic spaces of the two species.



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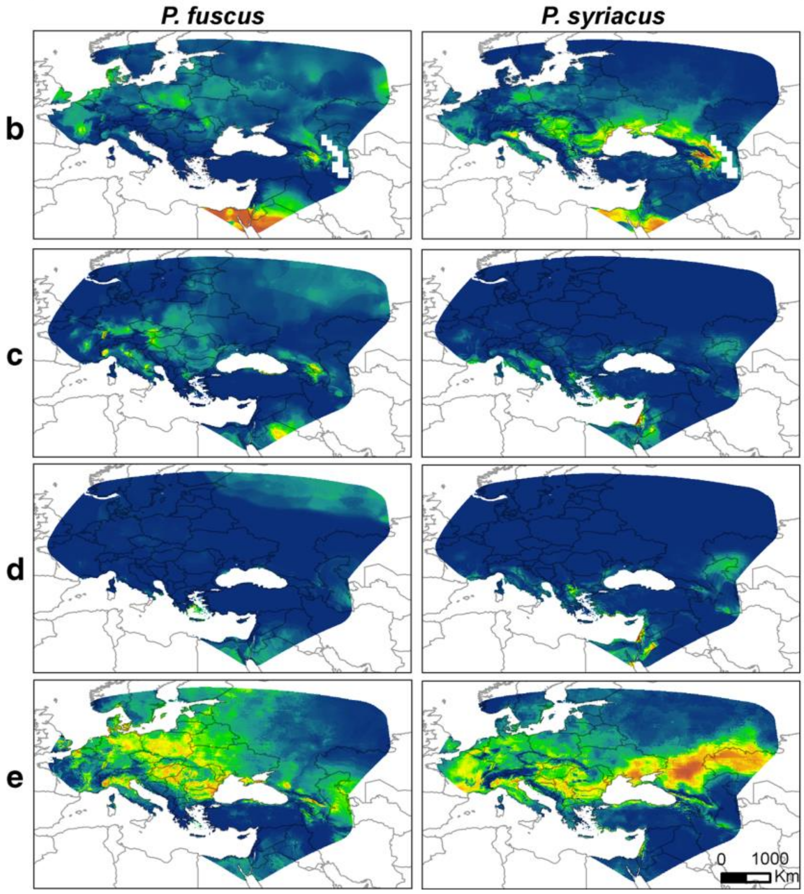

Figure 8 – The potential distribution of *P. fuscus* and *P. syriacus* in: (a) present climate for the two species, (b) Last Interglacial climate, (c) Last Glacial Maximum MIROC Scenario, (d) Last Glacial Maximum CCSM Scenario, and (e) A1B scenario for 2080. The modeling extent merges the minimum convex polygons of the two species and an additional buffer zone of 335 km that includes all fossil records.

GENERAL CONCLUSIONS

This thesis aimed to test the usefulness of spatial models in biodiversity management using amphibians and reptiles as case studies. In introduction I briefly presented what kind of spatial data are needed in biodiversity conservation, the research questions that can be answered using these data, and how important is their quality to sustain an effective management. With this background I evaluated the quality of the distribution data of amphibians and reptiles of Romania in Chapters 1.1, 1.2, and 1.3, whereupon I presented three case studies that show how spatial modeling is used at local scale (Chapter 2), regional scale (Chapter 3) and at the scale of species ranges (Chapter 4).

Chapter 1.1.

Spatial patterns identified in this chapter highlight a proper inventory of amphibians in central and western parts of Romania and an inventory gap in southern part. For reptiles, the spatial analysis highlights an intense inventory of the warmer and drier regions from south-east and south-west and a proper, uniform inventory across the rest of the country. These analyses provided an image of the inventory quality of amphibians and reptiles distribution in Romania.

Chapter 1.2.

At national level, Inventory Completeness of amphibians is positively related with altitudinal range, the index being high at higher altitudes (i.e., complete inventory of mountain areas) and low at lower altitudes (i.e., poor inventory of the plains), suggesting the mountains rather than the plains are more attractive for Romanian researchers. Inventory Completeness of amphibians is positively related with the surface of the protected lands, the index being higher when the protected surface is bigger. This result suggest that protected areas are over-sampled at national level detrimental of unprotected lands that can still shelter abundant amphibian populations (e.g., Danube Floodplains, Lower Course of Olt River, Bărăganului and Siretului Inferior Plains). Inventory Completeness of amphibians is negatively related with the distance towards cities where herpetologists are working. The inventory effort decreases if the distance towards these cities increases. This relation limits the quality and usefulness of national distribution databases.

Chapter 1.3.

In this chapter I report the presence of *P. syriacus* in 36 new locations in Romania. In Galati County I report the species with more than 40 km northwards of the last known locality. In Mehedinți County I reported its presence at 50 km westwards from the last known locality. Furthermore, the species was detected in many locations along the Danube Floodplains and Bărăganului Plain. We suggest the reason why these regions are under-sampled is the lack of interest of herpetologists given the agricultural landscape. Moreover, the results in this chapter suggest the agricultural landscape from southern Romania maintain viable populations of amphibians probably due to the presence of irrigation canals and nearby ponds and reservoirs. These results have implications for the conservation status of the species in Romania and European Union demonstrating the existence of spatial biases in national distribution maps.

Chapter 2.

The observed density of tortoises in the studied archaeological site increased from 5.1 in 2012 to 12.1 individuals per hectare in 2015, due to the higher sampling effort. Distribution of weight classes is dominated by individuals around approximately 1800 g. The population structure is as follow: 35.5 % females, 32.2% males (balanced sex ratio of 1.1), 12.7 %

subadults, and 19.4 % juveniles. All these results suggest that the tortoise population I have studied in this chapter is young and viable in the habitats modified by archaeologists.

Males and females were taking advantage on archaeological diggings as follows: males are abundant inside the diggings in early spring when temperature is still low; females are abundant inside the diggings in the nesting season; both sexes are abundant inside the diggings right before hibernation and during hibernation.

Finally, I highlighted the importance of a joint conservation approach for biodiversity of archaeological sites.

Chapter 3.

Pond losses and changes in terrestrial habitat in north-western France have different implications in the connectivity decline of amphibian populations. These implications differ from species to species: *A. obstetricans* and *T. cristatus* have to cope with longer distances during migrations because their reproduction ponds almost disappeared. When all species are analyzed as a whole the connectivity decline is related with changes of terrestrial habitats rather than pond losses.

I consider useful to restore and create new reproduction ponds, especially for species in significant decline. Still, I recommend minimizing the expansion of arable lands over pastures and costal dunes in future agricultural policies.

Chapter 4.

The present ecological niche models of the spadefoot toads showed a potential distribution that is extended outside the known range, southwards for *P. fuscus* and northwards and westwards for *P. syriacus*. These results suggest that their present distribution is not at equilibrium with the climate. The potential distribution in the Last Interglacial included British Isles and vast areas in west, center, and northern Europe for *P. fuscus*, and included northern Balkans for *P. syriacus*. Model testing based on fossil records showed a good predictive performance of these past projections. During the Last Glacial Maximum the suitable climatic space persisted only in few refugia in southern Europe, Pannonian Basin and Caucasus for *P. fuscus*, and Israel, southern Balkans and Caucasus for *P. syriacus*.

Present distribution models highlight a relatively low niche similarity between the two species, comparable with Last Interglacial. Still, the projection towards 2080 showed a significant increase of their niche similarity. This expected similarity has implications in protection and conservation of the syntopic populations of the two species. The contact zone of the two ranges is expected to be wider thus triggering competition disturbances leading to the local decline of the weaker species.

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