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Survey of the amphibians in "Fânațele Clujului – Copârșaie", part of the "Dealurile Clujului de Est" (ROSCI0295) Natura 2000 protected area

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Abstract. As habitat loss poses challenge to conservation, it is becoming increasingly important to address questions about the extent to which connectivity between habitat patches is changing, and how this affects the local population of different species in these patches. The objective of our research was to monitor ponds and the pond-breeding amphibian species in a protected area. Therefore, we conducted day and night surveys, and compare the data collected in 2022 with the results of the latest available survey (2019), to simulate the patch occupancy of amphibian species over a 25-year timeframe. We found that combining the species occupancy data collected from both day and night surveys lead to higher patch occupancy values and higher number of registered individuals, compared to data collected only during daytime. The number of ponds decreased from 2019 to 2022, and further habitat loss could result in the disappearance of the local population if the area continues to dry out. Climate and landscape change could be major contributors to habitat loss in the future, therefore, in order to ensure the persistence of these local populations, we recommend the development of climate and habitat scenarios, and the planning of conservation measures based on these scenarios.

Keywords: amphibian conservation, Transylvanian Plain, SPOM

Introduction

Engaging society to perform nature conservation activities in human-dominated landscapes is a challenge (Robinson, 2006). In Eastern Europe, the proximity of human-nature relationship resulted in landscapes with high cultural and biological diversity (Akeroyd and Page, 2007; Strohbach *et al.*, 2015). In these bioculturally valuable landscapes, the effectiveness of conservation can be enhanced by rethinking the role of humans and reconnecting them to nature by raising awareness to the natural values surrounding them (Grodzińska-Jurczak and Cent, 2011; He *et al.*, 2020, Ives *et al.*, 2018).

Scattered ponds are important parts of the mosaic of cultural landscapes that provide suitable habitats for many species, including amphibians (Calhoun *et al.*, 2017; Cogălniceanu *et al.*, 2012; Hartel and von Wehrden, 2013). From a conservation point of view, these ponds and the amphibian species inhabiting them deserve considerable attention because they are vulnerable elements of the landscape in the era of global change (Blaustein and Kiesecker, 2002; Nori *et al.*, 2015).

Nearly 40% of amphibian species are currently threatened with extinction, yet 53% of these species are distributed mostly outside protected areas (Bolochio *et al.*, 2020). Thus, the conservation of both protected and unprotected landscape features and landscape components that contribute to amphibian conservation is highly important.

According to Curado *et al.* (2011), between 1975 and 2006, the number of amphibian breeding ponds decreased as the grasslands around ponds decreased and the area of crop lands increased. The ponds surveyed in pastures were cattle ponds, which either were filled in or dried up due to land abandonment, resulting in the loss of amphibian breeding habitats, which affected the local amphibian populations (Curado *et al.*, 2011). A recent study showed that changes in a complex, multi-component socio-ecological system led to land abandonment, which resulted in an increase in the area's shrub cover and the drying out of a significant part of the ponds (Erős *et al.*, 2020).

Effective conservation measures require the continuous monitoring of habitats and the knowledge of species abundance and patch occupancy. In the case of amphibians, if data are recorded during multiple field visits, the changes in patch occupancy and probability of species detection can be traced over time (MacKenzie *et al*., 2002; MacKenzie, 2005). These can be important indicators of population and habitat changes (MacKenzie *et al*., 2002). Both field surveys and data analysis are needed to track differences between model and climate or environmental changes (Walls and Gabor, 2019).

The objective of our research was to repeat the survey of ponds and amphibians after three years in a protected area at the periphery of the Transylvanian Plain. Our specific objectives were threefold:

O1. To compare the number and area of ponds, as well as the occurrence of amphibians in ponds with data from 2019 reported by Erős *et al.* (2020).

O2. To construct incidence function models (IFMs) to describe pond profiles that can be occupied by amphibians in the future.

O3. To predict the patch occupancy levels of each amphibian species over a 25-year timeframe using stochastic patch occupancy models.

Materials and methods

Study area

Our study area was within the "Fânațele Clujului – Copârșaie" botanical reserve (46°50′28″ N, 23°38′31″ E), part of the "Dealurile Clujului de Est" Natura 2000 protected area (ROSCI0295). This area was a pasture with steppe vegetation elements grazed by cattle, buffalo and sheep (see Erős *et al.*, 2020). Temporary and permanent-like ponds (i.e., dry out only once in several years, in case of severe drought) were scattered across a 127-ha surface (Fig. 1). Eight amphibian species were reported from this area in the literature: *Bombina variegata*, *Rana dalmatina*, *Pelophylax* kl. *esculentus*, *P. ridibundus*, *Hyla arborea*, *Pelobates fuscus*, *Triturus cristatus*, and an endemic subspecies: *Lissotriton vulgaris ampelensis* (Erős *et al.*, 2020; Sos and Hegyeli, 2015).

Data collection

We conducted field surveys three times in 2022: 12 March, 04 April and 21 May. On each date, we surveyed amphibians at day and night (hereafter referred to as *survey types*). Easily accessible and small-sized ponds (area approx. 10-2000 m^2) were completely surveyed, while larger ponds (area >2000 m^2) were sampled close to the shore (ca. 1-3 m from the shore) at 3-5 sampling points. For some ponds were impossible to sample 3 m from the shore due to the deep mud. We measured the area of small-sized ponds using metric tape on the field, while the area of larger ponds was measured with GPS device (accuracy < 5 m). We took into account both individuals seen regardless of their stage of development, or heard vocalizing and identified based on their sound. Netting was used to increase the detection of species hiding in the mud, such as *Pelobates fuscus*. We assessed patch occupancy on a binomial scale: 1 if a species was present in a pond at least once during sampling, or 0 if a species was absent during samplings. At each survey occasion one person was responsible for performing the sampling activity, while a field assistant recorded the collected data on the OpenHerpMaps platform (openherpmaps.ro) using the OpenBioMaps mobile application.

Data analysis

To characterise the main changes in the study area in terms of amphibians and their habitats between 2019 and 2020, we compared the area of ponds and their occupancy by amphibians. Therefore, we performed Mann-Whitney U-test, the pond area data being non-normally distributed. We provide median and interquartile range as descriptive statistics about pond areas. To compare the amphibian occurrence in ponds, we calculated the percentage of occupied ponds and we compared these percentages.

Incidence function model (IFM) is a spatially realistic model, which uses the formulas of Hanski's (1999) metapopulation model and the probability of pond occupancy can be predicted. Using this model, we determined those pond area thresholds for each amphibian species, where the probability of occupancy is higher than 0.5. This model type contributes to species-specific conservation measures which can be focused also on different pond area ranges. The IFMs were implemented in the R environment (R Core Team, 2021) using the tutorial of Oksanen (2004). In this implementation IFM is a special generalized linear model with binomial error distribution, which uses the natural logarithm of habitat patches' size as independent variable and the occupancy state of habitat patches (1 if occupied, else 0) as dependent variable. The model takes into consideration the isolation of habitat patches by adding isolation values to patch areas as linear predictors (see Oksanen, 2004). The isolation of a habitat patch is given by: $\sum exp\ (-dij \times\ \alpha)pjAj$, where *dij* is the distance between patch *i* and *j* patch, α is the inverse of dispersal rate of the species (Tab. 1), *pj* is the occupancy state and *Aj* is the area of *j* habitat patch. Using IFMs we extracted pond area values with predicted occupancy probability at least 0.5 for each amphibian species and we compared them using Kruskal-Wallis H-test and Pairwise Wilcoxon Test with Holm *p*-value adjustment.

The Stochastic Patch Occupancy Model (SPOM) is also a spatially realistic stochastic model using colonization and extinction probabilities which simulates the pond occupancy over time. The model starts from occupancy states in timestep *t* and determines how the patch occupancy (i.e., presence-absence of the species) changes from time *t* to time *t+1*. If a patch of habitat was not occupied at time t, but is close enough to other patches occupied at time t, it is likely to be occupied at time t+1 based on the colonisation and extinction probabilities. The model requires the specification of colonization and extinction functions, therefor we assumed that colonization and extinction can be described by the formulas of Hanski (1994, 1999) (see formulas summarized in *MetaLandSim* R package documentation; Mestre *et al.*, 2016). We assumed that the area and number of habitat patches do not vary over time. This assumption is used to answer our third objective, highlighting how patch occupancy would change over a 25-year time frame if the study area remains in its current state. SPOMs were run 1000 times and the average of the results was plotted for each species.

Both IFM and SPOM models are necessary to take into consideration the species-specific dispersal distances. For our species dispersal distances are summarized in Tab. 1 based on Trochet *et al.* (2014).

Table 1. Dispersion distance of amphibian species considered in this study.

Species	Dispersal distance (m)
Rana dalmatina	1500
Bombina variegata	1200
Pelophylax complex	800
Hyla arborea	2200
Lissotriton vulgaris ampelensis	1000
Triturus cristatus	1000

Results

Pond characteristics, occurrence and number of individuals

15 ponds were identified during the three sampling occasions in 2022, 12 of which were identified in 2019 too. Of the 19 ponds identified during the survey performed in 2019, 7 did not form in 2022 (Fig. 1). Taking into account that 5 out of 15 ponds found in 2022 were permanent-like ponds, the temporary pond loss was 78% compared to 2009 data (n = 47) reported in Erős *et al.* (2020). The pond area ($median_{2022}$ = 160 m^2, IQR_{2022}: 78 – 1218 m^2) has not

changed since the 2019 survey (median$_{2019}$ = 176 m^2, IQR$_{2019}$: 66 – 290 m^2; Mann-Whitney U-test: W = 121.5, p-value = 0.48). All amphibian species known from the area were found, except *Pelobates fuscus*.

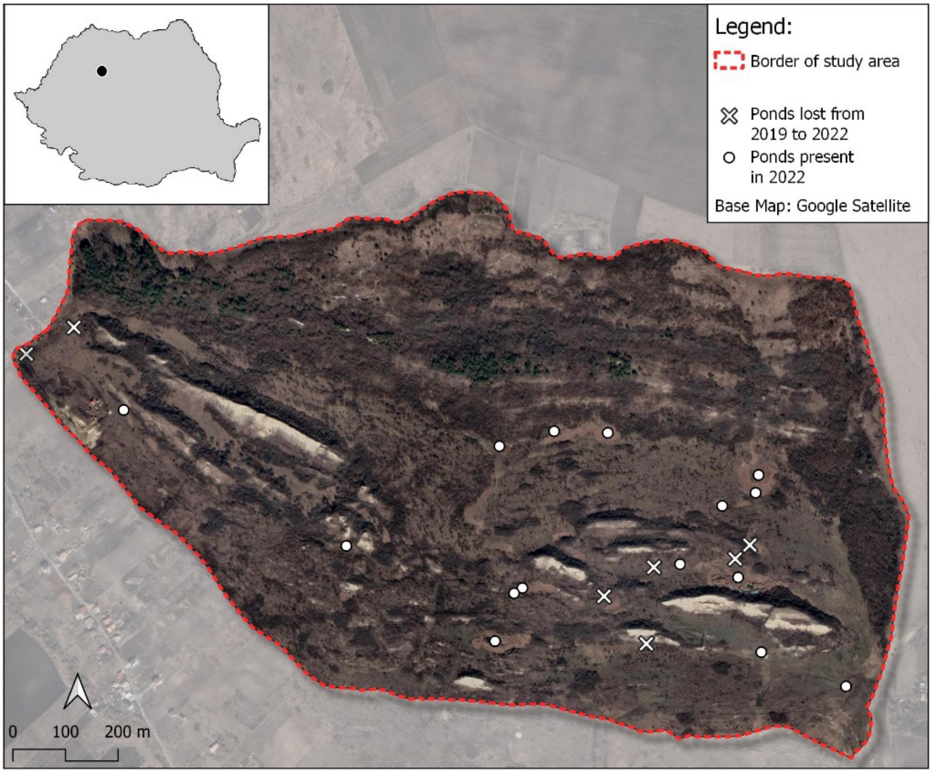

Figure 1. Map of study area. The black dot denotes the position of study area in Romania, while white dots denote ponds existing in 2022 and crosses denote ponds that existed in 2019 but dried out in 2022. The map was generated using Google Satellite.

In 2022, *Rana dalmatina* was found in the highest number of ponds, while *Hyla arborea* was found in the lowest number of ponds, and only during night-surveys (Fig. 2). Overall, day surveys supplemented with night surveys resulted in higher patch occupancy values for each species (Fig. 2). We found no difference in the total number of individuals between the day and night survey during the first (W = 33, p-value = 0.072; Fig. 2) and the last survey (W = 78, p-value = 0.144), but we found significantly higher number of individuals in the second day-survey (W = 114, p-value = 0.04; Fig. 2).

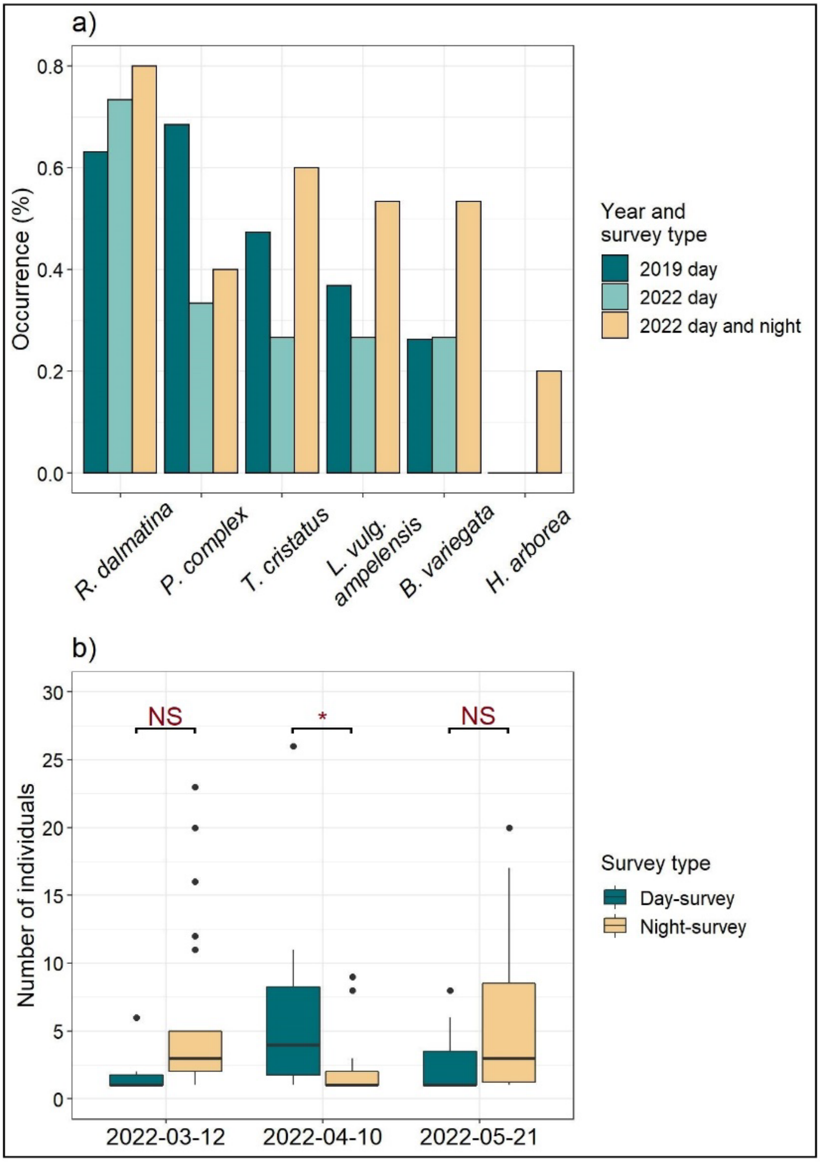

Figure 2. Panel a) amphibian species occurrence in ponds by survey year and type. We note that pond number in 2019 was 19, while in 2022 was 15; panel b) Cumulative umber of individuals in ponds by survey type, “NS” denotes non-significant differences between number of individuals by survey type, while “*” denotes p-value < 0.05 of Mann-Whitney U-test.

Incidence function models

Pond area was not found to be a significant factor of pond occupancy in the case of *Hyla arborea* and *Pelophylax* complex (Tab. 2). The model estimate of *Pelophylax* complex was unreliable, with lower estimate value than its standard error. Incidence function model estimates were similar in the case of *Triturus cristatus* and *Lissotriton vulgaris ampelensis* (Tab. 2).

Comparing pond area values by occupancy probability greater than 0.5, we found that *Rana dalmatina* and *Pelophylax* complex occupied significantly smaller ponds than other species (Kruskal-Wallis H-test: χ^2 = 29.8, df = 4, p-value <0.001; Fig. 4). *Triturus cristatus*, *Lissotriton vulgaris ampelensis* and *Bombina variegata* occupied ponds with similar area (Fig. 3). The IFM model did not predict occupancy probability larger than 0.5 for *Hyla arborea*, therefore this species was excluded from the comparison and is not presented in Fig. 3.

Table 2. Incidence function model estimates: the effect of pond area on occupancy. [a]p-value is presented if it is significant; "NS" denotes non-significant p-value.

Species	Estimate	SE	z-value	p-value[a]
Rana dalmatina				
Intercept	-25.5	2.2	-11.6	<0.001
log(Pond area)	1.34	0.48	2.8	0.005
Pelophylax complex				
Intercept	-18.37	1.1	-16.68	<0.001
log(Pond area)	0.035	0.2	0.18	NS
Triturus cristatus				
Intercept	-21.76	1.25	-17.4	<0.001
log(Pond area)	0.5	0.22	2.23	0.023
Lissotriton vulgaris ampelensis				
Intercept	-21.66	1.28	-16.92	<0.001
log(Pond area)	0.49	0.22	2.21	0.03
Bombina variegata				
Intercept	-21.59	1.27	-16.98	<0.001
log(Pond area)	0.44	0.22	2	0.046
Hyla arborea				
Intercept	-23.48	3.3	-7.12	<0.001
log(Pond area)	0.73	0.49	1.5	NS

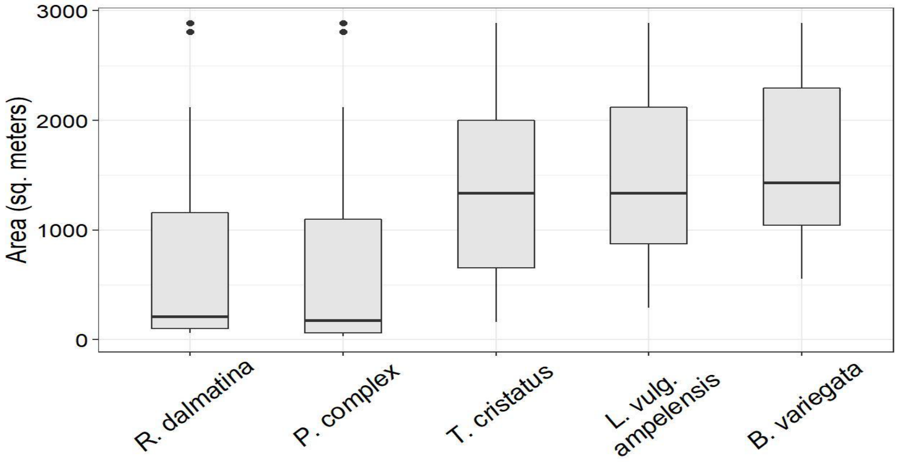

Figure 3. Pond area for species where predicted pond occupancy probability by incidence function models was greater than 0.5.

Stochastic patch occupancy models

Stochastic patch occupancy models showed that the patch occupancy of the species can increase over time, if the landscape will not change, i.e., the number of ponds and their area will not decrease (Fig. 4). During stochastic patch occupancy modelling, the highest patch occupancy was achieved by *Rana dalmatina*, while the lowest was achieved by *Triturus cristatus* and the two species belonging to the *Pelophylax* complex (Fig. 4).

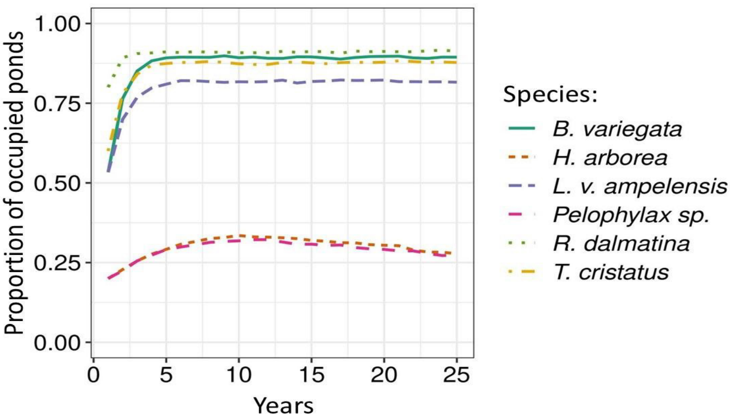

Figure 4. Results of stochastic patch occupancy models projected to a 25-year timeframe if the habitat patches and surrounding landscape remain in their current state.

Discussion

The data collected from a study area regarding the presence or abundance of target species depends largely on the survey method used to collect given data. Therefore, we surveyed our study area three times to get reliable data on pond occupancy and abundance of amphibian species inhabiting the study area. Twice in three surveys we found higher number of individuals at night surveys than during daytime. Combining day and night surveys is important, because the detectability of amphibian species may vary between years and species (see discussed in Schmidt, 2005), under different environmental variables (e.g., rainfall, temperature), even as the days of the year progress (Petitot *et al.*, 2014), and is highly dependent on the experience of the surveyor (Sewell *et al.*, 2010).

The incidence function modelling based on presence-absence data of species derived both from day and night survey showed that all ponds, regardless of size, are occupied by different amphibian species. *Triturus cristatus*, *Lissotrition vulgaris ampelensis* and *Bombina variegata* occupied with higher probability (larger than 0.5) ponds larger than 1000 m^2 (Fig. 3), while *Rana dalmatina* and species from *Pelophylax* complex occupied smaller ones. Unfortunately, the number of temporary ponds is in a constant decrease, i.e., less temporary ponds were formed in 2022 than in 2019, but the temporary pond loss comparing to 2009 data was 78% (REF). Therefore, conservation activities should address all pond types to ensure the persistence of all amphibian populations. Pond loss is recognized as a major threat to the amphibian population (Arntzen *et al.*, 2017; Cushman, 2006; Erős et al., 2020).

Successful creation and maintenance of ponds to conserve valuable aquatic habitats is a great challenge (e.g., Collserola Natural Park's project; Pinto-Cruz et al., 2017). The well-maintained ponds with longer hydroperiods have higher biodiversity in traditional landscapes (Hartel et al., 2014) and in urban environments as well (Beja and Alcazar, 2003; Oertli and Parris, 2019). In our study area, the increase in shrub cover over the last decade (Erős *et al.,* 2020) may be a major threat to pond formation and sustainability. The curbing of this vegetation growth could be an initial step in conserving ponds as in the case of *Epidalea calamita* frog (Buckley *et al.,* 2014; McGrath and Lorenzen, 2010). Another successfully deployed active conservation measure is the implementation of water pumps to actively maintain the hydroperiodicity of ponds (Mathwin *et al.*, 2021).

Based on our SPOMs, the under a constant landscape and compared to other species inhabiting our study area, the patch occupancy of the *Rana dalmatina* could be the highest of the studied species in the next 25 years. Our model accounts for an ideal state; however, long-term studies performed on this species have shown that population size fluctuations are influenced by stochastic conditions (e.g., weather) and population density (Hartel and Öllerer, 2009) or the presence of predators (Schmidt *et al.,* 2021). Indeed, small populations may be more susceptible to extinction due to stochastic events than larger populations (Pellet and Schmidt, 2005). The ideal state represented by our SPOM can be affected in several ways (e.g., changes in climate and habitat parameters, or in biotic interactions). Climate change has already resulted in the inclusion of several amphibian species from Australia on the IUCN Red List (Hero *et al.*, 2006), and might be a contributing factor to the loss of amphibian biodiversity in Europe as well (Popescu *et al.*, 2013). However, there are surprisingly few examples of specific climate change measures being integrated into regional or local amphibian management practices (Shoo *et al.*, 2011). Changes in habitat parameters (e.g., hydroperiodicity, temperature) or competition between amphibian species have negative impacts on the survival of species, and need to be accounted for during protected area management (Cayuela *et al.*, 2018; Roth *et al.*, 2016; Tournier *et al.*, 2017).

Conclusions

Day and night surveys can complement each other to provide a good quality snapshot of the amphibian species and their patch occupancy. In this study we show that the number of ponds, mostly the number of temporary ponds, decreases over time. Local populations of amphibian species can be conserved if there will be no significant negative changes in habitat and landscape, or if protective measures will be implemented. The continuation of small-scale monitoring activities and awareness raising regarding the disappearance of ponds are essential to protect and conserve the remaining local populations of amphibians in the protected area.

Data availability. All data and scripts are available by request by contacting the corresponding author via e-mail.

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