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INCORPORATING OCCUPANCY MODELS IN DESIGNING STUDIES OF ANIMAL DISTRIBUTION: A GLIMPSE ON THE HABITAT USE OF AN AMPHIBIAN IN THE SAXON LANDSCAPES OF TRANSYLVANIA

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SUMMARY. The importance of occupancy models in studying the habitat use and distribution of organisms was only relatively recently emphasized. Their advantage is that allow to predict the site occupancy and at the same time to quantitatively estimate the detection probability of the studied organism. Here we apply for the first time in Romania these models on Hyla arborea, a locally and regionally common but threatened amphibian. 30 permanent ponds were studied in 2007 and 2008. Our results show that the detection probability is high (>0.7), and the differences between the found percentage of occupancy and predicted occupancy was small. However, the data not accounted for detection probability may underestimate the use of ponds containing predatory fish. According to the count data there is a sharp decline of *H. arborea* in these ponds, but the occupancy models predict no such decline, suggesting that some ponds with H. arborea were missed in 2008. The detection probability was positively related to the emergent vegetation cover in the ponds, but the effect of vegetation was stronger in 2007 than in 2008. We suggest the estimation of detectability on different sensitive species before their local - regional decline.

Keywords: habitat, distribution, conservation, detection probability, *Hyla arborea*, Romania

Introduction

Knowing the site occupancy and distribution of organisms and also its spatial and temporal variation was and actually is an important challenge for ecologists. The range of application of such data is wide: biogeography (Bănărescu, 1970), island biogeography (MacArthur and Wilson, 1968), metapopulation ecology (Lewins, 1970; Hanski, 1998), community ecology (Simberloff, 2004), landscape ecology (Hartel et al. 2008), distributional change of organisms (Skelly et al. 2003) the ecology of invasive

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species (Simberloff, 2000), the climate change effects (Arajuo et al. 2006), biodiversity monitoring projects (Gibbons et al. 1997), conservation biology (Pellet and Schmidt, 2005) and environmental impact assessments.

When interpreting field data, researchers often assume that the detection probability of the organisms is 1 (i.e. the species, if present, is detected, otherwise not). However, a growing body of evidence suggests that the detection probability of 0 is rare in nature (Schmidt, 2004; 2005; MacKenzie 2005a;b), most of species having a detection probability less than 1. Therefore the site occupancy or demographic parameters of populations may be underestimated to an unknown degree if the detection probability is not taken into account. The costs of these biases may be high: local population turnovers (Moilanen, 2003), population trends (funk et al. 2003; Schmidt, 2004) may be misestimated, important habitats for organisms may be wrongly identified (Mazerolle et al. 2005), local and national status of organisms may be misestimated (Schmidt, 2004), infrastructural development may be wrongly planned and finally priorities for management and conservation may be improperly set (MacKenzie, 2005a;b; Schmidt, 2005).

As the studies regarding the herpetofauna inventories are flourishing in Romania (the first comprehensive results on a region were presented in Ghira et al. [2002]; and the numerous studies following that report for example Covaciu-Marcov et al. [2006], Strugariu et al. [2008]) our preliminary purpose is to attract attention to researchers to incorporate site occupancy models in their research design and data analysis. Researchers often use court data to express the proportion of habitat used by different species. However, the count data represent only an index of the true value of habitats occupied by the studied species (and not a "mirror" of it, as it is assumed) and are dependent on the detection probability and also on the real (but unknown) values of the measured parameter (Schmidt, 2003; 2004). The count data provide only a minimum estimate of an unknown quality (Schmidt, 2003): the researcher doesn't know how many specimens/populations he missed in his study. When the comparison of two or more counts is attempted (for example the proportion of habitat use in different years) the possibility for wrong conclusions becomes even higher because the detection probability and the true value of the habitats occupied may also change from year to year, to an unknown degree. As Schmidt (2003; 2004) noticed, amphibian ecologists seem to be unaware of pitfalls that the data unadjusted for detection probability represent. The first study that incorporated detection probability in estimating site occupancy rates in amphibians seems to be that of MacKenzie et al. (2002). Till then and since then many papers appeared with significant contribution on the field of amphibian ecology that not consider the detection probability in their study design (Brönmark and Edenhamn, 1994; Hecnar and M'Closkey, 1996a,b; Hecnar and

M'Closkey, 1997; Sjögren, 1991; Gagne and Fahrig, 2007 but see Mazerolle et al. 2005). The long term series analyses that were on the base of the "global decline" conclusion in amphibians (Houlahan et al. 2000) also used data that were not adjusted to detection probability. Our site occupancy studies on the Tarnava Mare basin are not exceptions (Hartel et al. 2006; 2007a).

In this study we will apply site occupancy model to estimate pond use and detection probability of the Tree Frog (Hyla arborea) in a small (30) sample of permanent ponds in the rural landscapes of the Saxon Transylvania (middle section of the Tarnava Mare basin). Hyla arborea is strictly protected under European (Bern Convention, Annex II, Habitat Directive, Annex IV) and Romanian level (Ministerial Order 1198/2005, Annex 3A). The population ecology of *H. arborea* is well studied in Europe (see for example Brönmark and Edenhamn, 1994; Vos et al. 2000; Pellet and Hoehn, 2004; Pellet et al. 2004; Grafe and Meuche, 2005; Rellet et al. 2005; Schmidt and Pellet, 2005; Van Buskirk, 2005; Vos and Stümpel, 1995; Pellet and Schmidt, 2005; Pellet et al. 2007; Kovács et al. 2007). Hyla arborea prefers shallow, sunny ponds (Pellet and Hoehn, 2004; Pellet, 2005) and it is sensitive to fish predation (Brönmark and Edenhamn, 1994; Van Buskirk, 2005; Hartel et al. 2007a) and habitat fragmentation (Andersen et al. 2004). Due to these features, it was proposed as umbrella species, its presence indicating amphibian communities that are species rich (Pellet, 2005; Öllerer, 2006). Many studies suggest that H. arborea is still widespread in Romania (for example: Ghira et al. 2002) including this area (Hartel et al. 2007a). Since the predatory fist introductions, together with other modification of the permanent pond habitats will expectedly be more and more frequent in Romania, monitoring the habitat use of this species in order to detect potential distributional changes at regional scale is urged. The specific aims of this paper are twofold:

- (i) To compare the naïve (i.e. count data) and estimated values of habitat use in *H. arborea* in two years (2007 and 2008) in two pond categories: ponds without predatory fish and ponds with predatory fish.
- (ii) To estimate the minimum number of site visits to conclude that the species is absent in the two pond categories.

Material and Methods

Study area and the surveys

The 30 permanent ponds surveyed for this study represented a small sample of previously surveyed (Hartel et al. 2007a;b) and newly located (2007) ponds. The study area is in the middle section of the Tarnava mare basin. The central section of the basin is dominated by hills ranging in elevation from maximum 600-800 m in the west to maximum 750-800 m in the east. The climate is continental (Pop 2001) with mean annual temperatures of around 6.5-9°C and mean annual rainfall ranges from 600 to 800 mm (Pop 2001). Other characteristics of the study site were previously presented (Hartel et al. 2007a,b).

In this study the presence of *H. arborea* in the studied pond was assessed using call surveys (Grafe and Meuche, 2005). The surveys were conducted in 2007 and 2008. Two of these surveys were conducted in May (between 10 and 18 of May) and an other survey was conducted in June (5-7). Only night surveys were made (i.e. between 20.00-22.00). The ponds were easily accessible with car from Sighisoara. We stay maximum 5 minutes listening for frogs at the edge of the pond (or at maximum 50 meters from it). Most of time the frogs were identified in less than one minute of listening. In this case, we stopped the listening in that pond and after noting the presence of *H. arborea* we continued the survey in the next pond. We are aware on the constrains caused by the short period of listening for calling frogs. Calling survey for relatively short time in optimal periods are frequently used in amphibian habitat use research (Pope et al. 2000, Gagne and Fahrig 2007 for example used 5 minutes as listening periods at each pond) and for us it was the best option to survey quickly the ponds for *Hyla arborea* in the two years.

Following Hartel et al. (2007a) we classified the permanent ponds in two categories: ponds without predatory fish and ponds with predatory fish. The fish species were included in predatory-non predatory category according to Hartel et al. (2007a). The emergent vegetation cover was quantified visually for each pond, as percentage (Hartel et al. 2007a).



The program PRESENCE (that implements the likelihood approach of site occupancy models developed by MacKenzie et al. 2002) was used to estimate the detection probability (p) and the proportion of sites occupied (ψ) . The essence of the site occupancy models developed by MacKenzie et al. (2002) is that they

simultaneously estimate the site occupancy, and detectability (see also MacKenzie 2005 a,b). The assumptions of this model (see also Schmidt 2005) are: (i) the sites remain occupied during the study period, no extinction, emigration or colonization happens, (ii) the detection probability is greater than zero and (iii) the detection of a species in a site is not influenced by the detection at other sites. Table 1 show a local example about how the detection histories should be used to estimate detection probability, by presenting the detection histories for N = 30 permanent ponds.

The detection probability (p) can be used to estimate the minimum number of visits $(N \min)$ necessary to be certain with a specified degree of confidence a species is absent from a surveyed site. The degree of confidence (α) for this estimation can be set to 0.05 (95% confident) (Kéry, 2002) or lower such is 0.01 (99% confident) (Reed, 1996). Thus



where p is the detection probability (see equation (1)). The equation for N min was solved for both 0.05 and 0.01 confidence intervals.

To calculate the probability of not seeing a species (F) after N visits following equation was used (Pellet and Schmidt, 2005):



F was calculated for every habitat type that we considered in this study, for N = 3 (i.e. the number of visits on each site, see above). We have calculated the above parameters (ψ , *p*, *N* min and *F*) separately for ponds that contained predatory fish and ponds without predatory fish (see above).

We calculated the rate of change in pond occupancy comparing the naïve estimates and the predicted estimates of the proportion of pond use in the two years. This was calculated as (site occupoancy₂₀₀₂-site occupancy₂₀₀₁)/site occupancy₂₀₀₁ (Schmidt 2005).

Table 1.

C: 4	Visi	Visits in 2007			its in 2(
Site	1st	2nd	3rd	1st	st 2nd	3rd	Status	
1	0	0	0	0	0	0	"absence"	
2	1	1	1	1	0	1	"persistence" 👞	
3	1	1	1	1	1	1	"persistence"	
4	1	1	1	0	1	1	"persistence"	
5	0	0	0	0	0	0	"absence"	
6	0	0	0	0	0	0	"absence"	
7	0	0	0	0	0	0	"absence"	
8	0	1	1	0	0	0	"extinction"	
9	1	1	1	1	1	L.	"persistence"	
10	0	1	0	0	10		"persistence"	
11	1	1	1	1	1	$\overline{21}$	"persistence"	
12	0	1	0	0	<u> </u>	0	"persistence"	
13	0	1	0	0**	\mathbb{Q}_1	0	"persistence"	
14	1	1	1		× ×1	1	"persistence"	
15	1	1	1 0	O	0	1	"persistence"	
16	0	0	0	\bigtriangledown_0	0	0	"absence"	
17	1	1		1	1	1	"persistence"	
18	1	10	1	1	1	1	"persistence"	
19	1	0	′ 1	0	0	1	"persistence"	
20	1	Ń	1	1	1	1	"persistence"	
21	1	1	1	0	1	1	"persistence"	
22	UN I	1	1	1	1	1	"persistence"	
23	~ 1	1	1	0	1	1	"persistence"	
24	2 0	1	0	0	0	0	"extinction"	
25	0	0	0	0	0	0	"absence"	
‴ 26	1	1	1	0	1	1	"persistence"	
27	1	1	0	1	1	0	"persistence"	
28	1	1	1	1	1	1	"persistence"	
29	1	1	1	1	1	1	"persistence"	
30	0	0	1	0	0	1	"persistence"	

The detection histories for *H. arborea* in 2007 and 2008. "0" = the species was not detected, "1" the species was detected

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Results

The detection histories for the 30 sites studied in the two years are presented in the Table 1. Two apparent extinctions and no colonization have occurred. Most of populations persisted from one year to the other.

Count data (naïve estimate of habitat use and distribution) The naïve estimate of the habitat use shows an overall decrease in this, from 80% (2007) to 73% (2008) (Table 2), the rate of change in pond occupancy being -0.27. The percentage of pond occupancy was larger in the ponds without predatory fish than in ponds with predatory fish. No decline was registered in the habitat occupancy in ponds without predatory fish (Table 2). The ponds with predatory fish showed a loss of 13.21% of pond populations from 2007 to 2008 (Table 2) suggesting quite large rate of change in the number of occupied ponds (-0.54).

Table 2.

The parameter	estimations of site	occupancy in <i>Hyla</i>	t arborea. See t	he abbreviations in the
	"Materials a	and Methods, The	model" section	l
		×	4	

	Naïve estimate	(SE)	р	<i>Nmin</i> (α = 0.05)	Nmin $(\alpha = 0.01)$	F (3 visits)		
2007	~	O'						
Ponds without predatory fish	1.00	1.00 (0.00)	0.96	1	1	< 0.0001		
Ponds with predatory fish	0.53	0.75 (0.27)	0.33	7	11	0.30		
All ponds	0.80	0.80 (0.07)	0.81	1	2	0.007		
2008								
Ponds without predatory fish	1.00	1 (0.00)	0.83	2	3	0.02		
Ponds with predatory fish	0.46	0.75 (0.36)	0.27	10	15	0.38		
All ponds	0.73	0.75 (0.08)	0.70	2	4	0.02		

 Ψ , p and N min

The Ψ also detect a decrease in the pond occupancy in 2008 compared to 2007 (Table 2) with a rate of change of -0.25. Similarly to the naïve estimate, Ψ showed a lower percentage of pond occupancy for the ponds with predatory fish, compared to the

ponds lacking predatory fish (Table 2). Contrary to the naïve estimation, the occupancy model showed no decrease in the occupancy of the ponds with predatory fish (Table 2). p was overall large for ponds without predatory fish (more than 70%) and small for the ponds with predatory fish (< 35%). Note that the p varied between the two years, being smaller in 2008. The values of *Nmin* suggest that 1-2 call surveys are enough to infer the absence of *H. arborea* from ponds (with 95% confidence). However, the ponds with predatory fish requires from seven to more than 10 surveys to infer the absence of *H. arborea* with the methodology presented here. The relationship between the p and the macrophyte coverage was positive (Figure 1). The percentage of variation explained by the reed cover was smaller in 2008 (24.7%) than in 2007 (31.5%) (Fig. 1).



Fig. 1. The effect of reed cover (%) on the detection probability of *H. arborea*. The upper figure represents 2007, the lower one 2008

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Discussion

We estimated the detection probability in the same sample of ponds for two years, thus, it was possible to assess its variation in the two years and also between pond types. This study suggests that there are very small (data for 2008) or no (2007) differences between the count data and estimated value of habitat use in the case of *H. arborea* in these landscapes. Similarly, Pellet and Schmidt (2005) found a large detection probability for *H. arborea* (p = 0.73) and also they concluded that with the sampling effort they use (average 3.7 visits per site) they detect all the populations in the studied area. In other, rarer species however, the differences between the naïve and estimated site occupancy were larger, especially in rare species. Thus, the naïve *vs* estimated site occupancies were 0.37 *vs* 0.48 in the case of *Bufo calamita* (p = 0.43), 0.11 *vs* 0.26 (p = 0.28) for *B. variegata*, but 0.11 *vs* 0.13 (p = 0.56) for *Alytes obstetricans* (Pellet and Schmidt, 2005).

In agreement with a previous study that used count data for 85 ponds (Hartel et al. 2007), the present study also suggest that the predatory fish negatively affect pond use by H. arborea (see also Brönmark and Edenhamn, 1991; Van Buskirk 2005) but the increased reed cover positively affect the pond populations of this frog. Nevertheless, in both years the use of predatory fish ponds was underestimated with only three surveys per pond in a season. Recent models show that detection probability depends on the population size fluctuations (Kéry 2002; Alpizar-Jara et al. 2004) also. Alpizar-Jara et al. (2004) demonstrated that the detection probability and the probability of extinction are negatively correlated. It is possible that the H. arborea population sizes in some ponds were decreased after the predatory fish introductions; therefore the calling activity was not so intense in the very small populations. Our own observations suggest that the chorusing intensity of *H. arborea* may sharply decrease in just 3-4 years after massive predatory fish introductions (Lepomis gibbosus, Perca *fluviatilis*, see further examples in Hartel et al. [2007]). As calling activity is an essential feature of the reproductive and spatial (i.e. metapopulation) dynamic of H. arborea populations (Vos and Stümpel, 2005), the negative effects of fish introductions may likely go beyond local populations to metapopulation. Bradford et al. (1993) have showed that the massive fish introductions may isolate populations of Rana muscosa in mountain ponds. The large choruses act as important conspecific "attractors" for *H. arborea*. Individuals may disperse up to 11 km distances to find already occupied ponds, avoiding empty ponds found on the dispersal route (Vos and Stümpel, 2005). As H. arborea also prefer ponds with temporary character (but more constant ones, which dry only occasionally), more emphasis should be given to the

creation and maintenance of such ponds in the surroundings of permanent ponds (Hartel et al. 2007b).

The status of a species requires knowledge about the trends in its population sizes (Houlahan et al. 2000) but also the trends of the number of populations (Sjögren 1991, Hecnar and M'Closkey, 1996). This study suggests that detection probability should be considered in determining status and trends of amphibian populations; otherwise the possibility to misestimate these aspects is high. The count data may suggest a sharp decline in pond occupancy from one year to the next but according to the occupancy model no such trend is obvious (see the ponds containing predatory fish in the Table 2).

The site occupancy models allow the use of many site and sampling specific covariates for accounting the detection probability: weather conditions (Pellet and Schmidt, 2005), habitat features (MacKenzie et al. 2002, Schmidt 2005, Mazerolle and et al. 2005, Pellet and Schmidt 2005), survey methodology and effort (Kéry, 2002; Bailey et al. 2004), season (Kéry, 2002; Kéry et al. 2005), population size (Kéry 2002). In this study we omitted many other possible effects may potentially act on the detectability *H. arborea*. We have "standardized" our surveys by making the site visits under weather conditions that we considered as being favorable for *H. arborea*.

The results of this study raise the question: how accurate were the count data presented in Hartel et al. (2007a)? The surveys in this area begin many years ago, and up to five visits were made on each pond in the activity period of this frog. Moreover, the searches on each sites lasts up to one hour in many cases (instead of five minutes of calling surveys). Assuming a variable but high (>0.7) detection probability (as found by this study) we are confident that the data analyzed in that paper (Hartel et al. 2007a) are not biased. However, more interesting is the situation of species that are locally rare in this area (*R. arvalis, B. viridis*). We believe that special survey programs should be planned for these locally and regionally rare species.

Conclusions and recommendations

Understanding the principles of site occupancy models will undoubtly make researchers more aware regarding the design of the studies and more efficient in allocating effort, nevertheless may help researcher to better formulate the objectives of his her study. As MacKenzie and Royle (2005) wrote: "A good study objective should be explicitly linked to how the data will be used to discriminate between scientific hypotheses about the system or how the data will be used to make management decisions". Accounting for the detection probability (and other parameters that can be estimated from this) is especially important in Romania because of the wide range of

natural-seminatural landscapes of which biodiversity is relatively poorly known. As the biodiversity assessment requires a huge amount of effort (financial, personnel or other), and time, the biodiversity and the organisms' distribution may be strongly underestimated. Site occupancy models allow researchers to estimate these biases in quantified way. Moreover, the potential loss caused by the always growing infrastructural, agricultural, urbanistic (or other) developments may also be estimated using these models (i.e. by estimating the likelihood of not finding a species in a landscape after a given number of surveys). When a national program is promoted to assess the distribution of a certain species in Romania, researchers may gather a good image on the organisms' detection probability and estimated site occupancy in different landscapes of Romania. With care, these results can be extrapolated for wider (but structurally similar) areas and represented on the maps using GIS. In this way the status of the species will be more accurately estimated and decision makers will have a clear image about the risks that development poses to biodiversity in different landscapes/areas of Romania.

Hyla arborea is a good candidate for monitoring studies that aims to explore distributional changes on habitat use caused by anthropogenic impact. This is because it is easy to be identified in the field (i.e. using call surveys), it is widely distributed in different regions of Romania (Ghira et al. 2002, Covaciu-Marcov et al. 2006, Strugariu et al. 2008), locally may be still abundant and extremely sensitive to fish introductions. Considering the fact that the spatial extent of habitat use and the detection probability are variable, it is preferable to estimate detectability of species that are sensitive to human induced changes in habitat quality before their local – regional decline (Hecnar and M'Closkey, 1997; Reed 1997).

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