



Species richness - pond area relationships of amphibians and birds in two Natura 2000 protected areas of Romania

T. Hartel^{1,3}, C. I. Moga², A. David² and I. Coroiu²

¹ Mihai Eminescu Trust, Str. Cojocarilor 10, 545400 Sighișoara, Romania

² Biology & Geology Faculty, Babes-Bolyai University, Str. Clinicilor 5–7, 400006 Cluj-Napoca, Romania

³ Corresponding author: T. Hartel. E-mail address: asobeka@gmail.com

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Abstract: In this paper, we present regional differences in pond area by focusing on species - pond area relationships between two groups of vertebrates differing in life cycles and ecological requirements: amphibians and birds. The study was conducted in two regions of Romania: the Târnava Basin and the Fizeș Valley. Ponds from Târnava were more vegetated with emergent aquatic plants (*Phragmites australis* and *Typha* sp.). The amphibian species richness in ponds was higher in Târnava than in Fizeș and, conversely, Fizeș contained a higher number of bird species. The diversity of the amphibian species is not related to pond area, however, there was a positive relationship in both regions between amphibian species richness and the percentage of emergent vegetation cover. Bird's species richness, on the other hand, was positively related to both pond area and vegetation cover in Târnava whereas only to vegetation cover in Fizeș. The z values of the species–area relationship for amphibians were low in both regions and slightly negative in Târnava. In case of birds, the z value was larger in Târnava than in Fizeș, suggesting that the number of species increased more with pond area in Târnava than in Fizeș.

Nomenclature: Duellman (1993), for amphibians; Sibley and Monroe (1990), Monroe and Sibley (1993) for birds.

Introduction

The relationship between the number of species to area and the underlying biological mechanism have long been in the focus of ecological research. The first work that presented this relationship with clear and testable working hypotheses was the theory of island biogeography by MacArthur & Wilson (1967) (see also Simberloff 1974). The theory of island biogeography quickly became the most important paradigm in ecology. Due to the recognition that habitat loss and fragmentation creates insularity and the number of species of a certain group is related to the “island” (=habitat fragment, patch) area (Usher 1987), the theory was soon applied in terrestrial habitats (Usher 1987, Lomolino 2000; reviewed by Watling and Donnelly 2006) and natural reserve design (Diamond 1975, Higgs 1981). In this approach, habitats are considered to be patches of different size and shape with edges that are easily delimited from a surrounding hostile environment (the matrix) (Lomolino 2000, Haila 2002, Fischer et al. 2004). Because the term “habitat” is a species-specific concept (Fischer and Lindenmayer 2007), the predictions of island biogeography should be applied only to those groups of organisms that strictly depend on a certain habitat type (Usher 1987). However, there are many organisms that depend on multiple patches rather than one habitat type (Watson 2002). For such cases, the term “landscape complementation” was introduced (Dunning et al. 1992). A landscape with high complementation may sustain more species with complex habitat requirements than a landscape with low complementation (i.d. Hartel et al. 2008). However, the spe-

cies richness of organisms that can be predicted by landscape complementation may not increase with the area of a single patch, as predicted by the theory of island biogeography (Cook et al. 2002, Watson 2002).

With the above considerations in mind, this study explores the species number and pond area relationships in two groups of vertebrates: amphibians and birds in two regions of Romania: the Târnava Mare basin and Fizeș Valley. The permanent ponds and the species groups considered are good candidates for testing both the the predictions and the weaknesses of the island biogeography theory because: (i) pond habitats are easy to distinguish from the surrounding terrestrial matrix (Céréghino et al. 2008); (ii) pond breeding amphibians require high levels of landscape complementation for their survival and reproduction, as their life-cycles depend on both aquatic and terrestrial environments; and (iii) water birds are strictly connected to the aquatic habitats, thus the pond-island approach may be useful to predict their species richness. Moreover, since both groups depend on the microhabitats offered by macrophyte vegetation cover, quantifying this parameter may also be used to predict their species richness in the ponds. The two areas where the studies were conducted were recently designated as Natura 2000 areas. Natura 2000 is the European Union's network of protected areas, with the aim to protect and conserve the most seriously threatened habitats and species. These habitats and species are listed in annexes of the Habitats and the Birds Directive. Comparative studies on similar groups may contribute to the elaboration of region-landscape specific management plans for biodiversity in these newly designed sites.

Materials and methods

Study areas

The middle section of Târnava Mare Valley (hereafter Târnava) is a hill area (the hills ranging between 600-800 m in the West to 750-800 m in the East). Temperature values decrease from an annual average of 9°C in the west to one of 6.5°C in the east. The regulations of the Târnava river and dam building along its tributaries during the last 40 years have generated a number of ponds along the valley (Hartel et al. 2007). We selected 33 permanent ponds in an area of approximately 2600 km² for this study.

The Fizeş Valley, located in the central part of Romania, is comprised of a hill area with hills ranging from 400 to 550 m, 12 large fish-ponds created in old marsh areas of the valley, and four fish-ponds along the Fizeş tributaries. The only natural pond from Transylvania, and also the deepest in the Valley, is the Pike Lake, also located on one of the Fizeş tributaries. All other ponds are man-made. Overall, 16 ponds were studied in an area of approximately 1200 km² of the Fizeş Valley (Sorocovschi 2005).

Data collection

The studies on amphibians were carried out regularly from 2000 (Hartel et al. 2006, 2007). Amphibians were inventoried by searching for eggs, dipnetting for both adults and larvae, direct observations of the active adults, and the detection of calling anuran males. Two to three surveys were made on each pond during the amphibian's reproductive period (March until mid-May) and another two to three surveys for larvae were carried out on each pond until the end of July (Hartel et al. 2007). The eggs were identified visually while walking along the ponds shoreline and the larvae by dipnetting. Each pond was studied for one hour. If any life stage of a certain amphibian species was identified, we considered the species present; otherwise the species was deemed absent. We are confident that this sampling effort allowed for the identification of all amphibian species that used the studied pond for reproduction.

The surveys for birds were made from the first week of April to the first week of July (2002 – 2006) in clear weather conditions, using binoculars and scopes (Koskimies and Vaisanen 1991). In each reproductive season, four observations per pond were made – two in the morning between sunrise and 11:00 a.m. and two in the late afternoon, approximately two hours before sunset. The line transect method was used along the shore of the ponds and reedbeds and point counts (with 50 m radius, 10 min/each sampling occasion) in the reedbeds (Bibby et al. 2000; see also Douglas et al. 2006, for details regarding the reedbeds). In the case of the ponds with large emergent vegetation patches, the points were randomly chosen to cover a minimum of 25 percent of each microhabitat from the emergent vegetation. Only the territorial breeding birds were considered in this study, that is birds displaying territorial behaviour or seen with chicks.

We measured three wetland parameters: the pond area (ha); the percentage (%) of emergent vegetation cover (*Phragmites australis* and *Typha* sp.); and the area of the emergent vegetation [m²]. All three parameters were deemed potentially important for both groups of species that we studied. Vegetation cover was estimated visually by two independent observers. Since vegetation cover may change yearly due to natural dynamic events or human disturbance, we used the vegetation cover data from the same year in which the estimations of amphibian and bird species numbers were made to control for these changes.

Data analysis

After the surveys were finished, we created a dataset that contained the cumulative number of amphibian and bird species for each pond. We used the species richness because this is the response variable in island biogeographic studies (MacArthur and Wilson 1967). Mann-Whitney U test was used to compare wetland parameters and species richness in the two regions.

One establishment of the theory of island biogeography (Arrhenius 1921, MacArthur and Wilson 1967) holds that there is a positive relationship between the number of species (S) and island area (A). This relationship can be described as a power model:

$$S = C \cdot A^z,$$

where C and z are constants. The power model is typically used when island-like habitats (high contrast patches, *sensu* Watson 2002) are sampled (Sheiner 2003). The power model is commonly approximated by taking the logarithm of both sides to allow linear regression estimation:

$$\log S = z \log A + \log C.$$

Since in Târnava there were ponds without birds, we used the transformation $\log(S + 1)$.

A multiple regression model was built using the pond area and the emergent vegetation cover as predictors and the estimations of species richness as dependent variables. In these analyses, the log transformed data were used. Due to the high collinearity between the pond area (ha) and the area of the emergent vegetation (ha) ($r > 0.6, p < 0.05$), only pond area and the percentage of emergent vegetation cover were used in the regression analysis.

Results

The ponds from the Fizeş Plain have a larger area (median [Min – Max] = 26.6 ha [3-130.8], SD = 36.39) than those from Târnava (median: 1.5 ha [0.02-270], SD = 55.97) (Mann – Whitney U test, $Z = -4.53, p < 0.0001$). The percentage of emergent vegetation cover was significantly larger in Târnava ponds (median: 35% [0.1 - 85], SD = 20.39) than in the Fizeş (median 4.6% [1-51], SD = 11.76) ($Z = 3.90, p < 0.0001$).

Table 1. Descriptive statistics of amphibian and bird species richness in the two areas. The *Rana esculenta* complex was treated as one species in both areas. The sample sizes were 33 ponds in Târnava and 16 ponds in Fizeş.

	Median of species richness	Minimum	Maximum
Târnava			
Amphibians	5.93	1	11
Birds	2	0	21
Fizeş			
Amphibians	5	3	7
Birds	16.5	8	22

Table 2. The multiple regression analysis of the relationship between the pond habitat variables and the species richness of amphibians and birds in the two areas.

	$\beta (\pm SE)$	<i>t</i>	<i>P</i>
A. Târnava			
Amphibians			
Percentage of macrophyte vegetation	0.84 (0.09)	8.84	<0.0001
	$R^2 = 0.71, F_{1,31} = 78.25, P < 0.0001$		
Birds			
Pond area	0.86 (0.12)	6.73	<0.0001
Percentage of macrophyte vegetation	0.34 (0.12)	2.69	0.01
	$R^2 = 0.60, F_{2,30} = 22.75, P < 0.0001$		
B. Fizeş			
Amphibians			
Percentage of macrophyte vegetation	0.52 (0.22)	2.32	0.03
	$R^2 = 0.27, F_{1,14} = 5.38, P = 0.03$		
Birds			
Percentage of macrophyte vegetation	0.73 (0.18)	4.18	0.001
	$R^2 = 0.54, F_{1,14} = 16.77, P < 0.001$		

Nine amphibian species were found in Târnava and six in Fizeş (the complete list of the identified species of the two vertebrate group can be seen in Appendix 1). We found no significant difference between the species richness of amphibians in the two regions (Mann – Whitney U test, $Z = -1.48$; $p = 0.13$) (Table 1). The multiple regression shows that there is no significant relationship between the area of ponds and the amphibian species richness in the two areas. The macrophyte cover, however, had a significant positive effect on the amphibian species richness both in Târnava and Fizeş (Table 2). The z and C values for the species area regression estimated for Târnava were -0.08 and 5.50 , respectively. In the case of Fizeş, the z was estimated as 0.11 and the C as 1.09 (Table 3).

The wetland bird species richness is significantly larger in Fizeş Plain than in Târnava (Table 1) ($Z = 4.96$, $p < 0.001$). In Târnava there were ponds not used by birds. These ponds were significantly smaller (median 0.1 ha, Min – Max = $0.02-1$, $n = 15$) than the used ones (median 2.2 ($0.1-270$)) (Mann - Whitney U test, $Z = -3.41$, $p = 0.006$, $n = 19$). No significant difference was found, however, in the percentage of macrophyte vegetation cover between the occupied and unoccupied ponds (occupied median: 23.33 ($0.3-80$), unoccupied: 18 ($0.1-66$), $p = 0.20$). According to the multiple regression (Table 2), both pond area and macrophyte vegetation cover were significant predictors for bird species richness in Târ-

Table 3. The values of C and z of the species number and pond area in amphibians and birds in the two studied areas.

	C	z
Târnava		
Amphibians	5.50	-0.08
Birds	2.86	0.30
Fizeş		
Amphibians	1.09	0.11
Birds	2.27	0.15
Pooling amphibians and birds		
Târnava	9.03	0.09
Fizeş	12.6	0.14

Table 4. Multiple regression analysis of the habitat variables and the number of amphibian and bird species pooled together.

	$\beta (\pm SE)$	<i>t</i>	<i>P</i>
A. Târnava			
Pond area	0.76 (0.11)	6.75	<0.001
Percentage of macrophyte vegetation	0.81 (0.11)	7.21	<0.001
	$R^2 = 0.69, F_{2,30} = 33.87, P < 0.001$		
B. Fizeş			
Percentage of macrophyte vegetation	0.74 (0.17)	4.14	<0.001
	$R^2 = 0.55, F_{1,14} = 17.21, P < 0.001$		

nava. These two variables explained 60% of the variance of the bird species richness in these ponds.

In the Fizeş, the vegetation cover was the only significant predictor of bird species richness (Table 2). The z and C values of the species area relationship in birds for Târnava were 0.30 and 2.86 and for Fizeş were 0.15 and 2.27 (Table 3).

Only the macrophyte cover was found to be an important predictor for the two vertebrate groups together in Fizeş (multiple regression, Table 4). However, both pond variables were important predictors for amphibian and bird species richness pooled together in Târnava (Table 4). In Târnava, the slope of species-area relationship for the species richness of both groups was $z = 0.09$ and $C = 9.03$. For Fizeş the z and C values were 0.14 and 12.6 , respectively (Table 3).

Discussion

Our results can be summarized as follows: (i) there was no relationship between the species richness of amphibians and the pond area, however the aquatic vegetation is a good predictor of amphibian species richness in both studied landscapes; (ii) bird species richness can be significantly predicted by both pond area and aquatic vegetation cover (%) in Târnava while in Fizeş only the aquatic vegetation is an important predictor for bird diversity; and (iii) when pooling together the species richness of amphibians and birds, a significant reduction of the slope of species number–pond area relationship was observed in Târnava.

The larger area of the fishponds from Fizeş than those from Târnava can be explained through the origin of the

ponds and the geomorphological properties of the areas where they are situated. Târnava basin is a hilly area through which the Târnava Mare river runs and the very narrow adjacent valleys where the tributary system is situated. The flooded areas are narrow, ranging from 50 to 100 m (Pop 2001). These flooded areas, together with the small (and in some cases temporary) character of the springs, are not suitable for the creation of large lakes with the exception of the Brădeni fishponds which are situated in the flooding area of the Hârtibaci Valley, with a large flood basin (up to 1.5 km) (Pop 2001). The fishponds from Fizeş are formed along the river Fizeş which was considerably larger in the past (flooded areas having up to 1 km width) (Săndulache 1968). The large ponds that have been created by dams function partly as fishponds and partly as irrigation systems for the agricultural lands (Sorocovschi 2005).

Pond size may not be an important predictor for the amphibian species richness for a number of reasons. Firstly, amphibians are not entirely aquatic organisms. An increasing number of studies demonstrate the importance of the terrestrial habitats in predicting pond occupancy of amphibians (see, for example, Gagne and Fahrig 2007). Secondly, amphibians do not use the interior section of ponds that are distant from the shore zone. This may be because the ponds are more exposed to fish predation. Personal observations (Hartel 2008 and unpublished results from Brădeni fishponds, Târnava) show that the majority of species rarely deposit eggs at a distance of more than 10m from the shore, even if those parts are well vegetated. Thirdly, due to predation risk, it is safer to be close to the water line, in the shallow part of the littoral zone when metamorphosis occurs. It is likely, therefore, that the increase of this parameter (i.e., the habitats offered by the shoreline of the ponds) would be the most important variable for amphibians (see below). If the terrestrial areas are not available for the newly metamorphosed juveniles (i.e., because the metamorphosis is completed far away from the shoreline), this may lead to the death of juveniles for some amphibian species (Hartel personal observations). However, it was demonstrated that the amphibian species richness in the individual permanent ponds is significantly higher than in the temporary ponds in Târnava, and was explained as a result of the larger complexity of the permanent ponds (Hartel et al. 2006). The habitat complexity in permanent ponds was related to the percentage of the emergent vegetation cover in this study. A previous study carried out in eighty-five ponds in Târnava (Hartel et al. 2007) showed that the emergent vegetation cover was an important determinant of the pond use of six amphibian species (*Triturus cristatus*, *Triturus vulgaris*, *Bombina variegata*, *Hyla arborea*, *Rana dalmatina* and *Rana temporaria*). Moreover, the species richness had a significant positive association with the percentage of emergent vegetation cover. Emergent vegetation cover grows in the shallow, productive parts of the ponds and provides good habitat refuge from predator fish (Holopainen et al. 1997). The emergent vegetation may also represent proper support for eggs in many species that breed in early spring (*R. dalmatina*, *R. temporaria*, *Bufo*,

bufo, *T. cristatus*) (Hartel 2008 and personal observations). While reed cover explains 71% of species richness variation in Târnava, it only explains a small variation of species richness in Fizeş (27%). This aspect needs further exploration.

In accordance with the findings of Oertli et al. (2002), our study show low levels of z for the species richness of amphibians in permanent ponds (Oertli et al. found values of -0.005 for 80 ponds and 0.0004 for a subset of 60 ponds in Switzerland). The slightly negative values of z in our study may indicate impoverishment of the quality of habitat with increasing area (Connor and McCoy 1979). This result was expected in Târnava, since the high density of predatory fish in the large fishponds could be detrimental for amphibians (Hartel et al. 2007).

The pond area was a significant predictor for the bird species richness in Târnava but not in Fizeş. The ponds from Fizeş are larger than those from Târnava (with a smaller standard deviation that suggests lower variation in the area) and also contain significantly more bird species. The ponds from Fizeş are large enough to support bird communities rich in species, and the increase of pond size probably does not add many more species to the community (as indicated by the low z value).

The slope of the species-area relationship can be decreased by high immigration rates (MacArthur and Wilson 1967) and low extinction/emigration rates (Johnson and Simberloff 1974) or a combination of these two (reviewed by Connor and McCoy 1979). A low extinction/emigration rate can be suspected in the Fizeş area due to the large pond areas and high amount of emergent aquatic vegetation cover (see Results) which in turn assures a safer habitat for nesting for most birds. It was experimentally shown that in larger areas, larger populations that are less prone to extinction can be established (Simberloff 1976). The low extinction/emigration rate leads to a larger number of species in larger areas. In Târnava, both the area and the macrophyte vegetation cover are important for wetland birds. If a high immigration potential is assumed, the increase in wetland area leads to a significant increase in bird species richness. The result is a steeper slope of the species-area relationship in Târnava compared to Fizeş. The ponds not used for breeding by birds in Târnava have a significantly smaller area than those used, yet no significant difference was found between the percentages of the emergent vegetation cover in the two pond categories. This confirms the importance of the pond area for bird species richness in Târnava. However, it should be mentioned that these ponds are occasionally used by different bird species for feeding and passage (e.g., *Fulica atra*, *Gallinula chloropus*, *Tachybaptus ruficollis*, *Ardea purpurea*) (Moga and Hartel, personal observations). This suggests that the small ponds may play an important, though occasional, role in the regional population dynamic of the birds. Emergent macrophyte vegetation, such as reed (*Phragmites* sp.), represents an essential breeding habitat for many rare bird species of Europe (see Tucker and Heath 1994, Naugle et al. 2001, Barbraud et al. 2002, Martinez-Vilalta et al. 2002,

Báldi 2004) and North America (see Riffell et al. 2001). Furthermore, as shown by the studies mentioned above, other factors, such as the density, structural diversity of the aquatic vegetation and temporal fluctuation of the water level are also important determinants of the bird species richness in ponds. These habitat features increase the microhabitat availability (Riffell et al. 2001), allow the coexistence of closely related species (Catchpole 1973, Amarasekare and Nisbet 2001), and create habitats safe from predators (Barbraud et al. 2002).

In conclusion, our results show that life history and ecology of groups and species should be considered in species-area relationships. Patterns of species-area relationships may be region specific, reflecting the differences between the habitat-landscape characteristics.

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Appendix

The list of amphibian and bird species identified as breeding in the ponds studied in the two areas. The file may be downloaded from the publisher's web site at www.akademai.com.