

M. Vences, J. Köhler, T. Ziegler, W. Böhme (eds): Herpetologia Bonnensis II.  
Proceedings of the 13th Congress of the Societas Europaea Herpetologica. pp. 47-50 (2006)

# The influence of habitat characteristics on amphibian species richness in two river basins of Romania

Tibor Hartel<sup>1</sup>, László Demeter<sup>2</sup>, Dan Cogălniceanu<sup>3</sup>, Mirela Tulbure<sup>4</sup>

**Abstract.** We present the distribution and aquatic habitat use of amphibian communities in two river basins of Transylvania (Romania) on a medium spatial scale: the middle section of Târnava Mare Valley and the Upper section of the Olt Valley. We used between 13 and 15 variables to characterize the breeding ponds and their surrounding terrestrial areas. A total number of 14 amphibian species and a species complex were identified in the two areas. In Târnava, the average species richness is larger in permanent ponds than in temporary ones. No significant differences were found between the species richness in permanent and temporary ponds in Ciuc. In permanent ponds of both areas the presence of high traffic roads in the vicinity (i.e. within an 800 m radius) explained more of the variation in species richness than the other habitat factors, whereas the presence of dirt roads accounted for the highest variation in species richness in temporary ponds.

## Introduction

Recent studies show that amphibians are in global decline (Houlahan et al., 2000, Stuart et al., 2004). The causes of this phenomenon are very diverse (see Corn, 2000 for a review). Habitat reduction caused by destruction and fragmentation is often cited as being the most significant anthropogenic cause of amphibian declines in Europe (Corn, 2000; Stuart et al., 2004). Understanding habitat requirements and habitat use of different species is a major challenge in conservation biology. Species with complex life cycles such as pond breeding amphibians need special attention because of the spatial heterogeneity of the habitats they require for living (Dodd and Smith, 2003). Romania still has extensive areas with minimum impact from human activities. We focused on two areas (Târnava Mare and Upper Olt Valleys) which have a high diversity of aquatic habitats that support species rich amphibian communities (Hartel et al., 2005; Demeter et al., 2006). There is little background information about the habitat availability and habitat use of amphibians in these two areas. Our aims during this study were: (i) to inventory habitat diversity and habitat use by amphibians and, (ii) to identify the most important anthropogenic factors influencing the amphibian species richness in the areas investigated.

## Materials and methods

The Târnava Mare and the upper Olt valleys are situated in central Romania. An area of approximately 101 km by 26 km was studied in the middle section of the Târnava Mare valley, and an area of 70 km by 25 km in the upper Olt valley (Ciuc Basin). The middle section of Târnava Mare Valley (hereafter Târnava) is dominated by hills with an altitude of 600-800 m, although the lower hills (600-750 m) are only found to the west of the valley. Temperature values decrease from an annual mean of 9 °C in the west to an annual mean of 6.5 °C in the east. The upper Olt valley (hereafter Ciuc) is a mountain basin in the Eastern Carpathians. The mean altitude of the basin is 700 m, and the surrounding mountains have an altitude of 1000-1800 m. Yearly average temperatures are between 1-4 °C in the mountains and 3.7-7.5 °C in the basin. The study period was from 2000-2004, with the great majority of the ponds being surveyed in 2004 in both areas. Amphibians were detected by dipnetting (both adults and larvae), torch count at night, and call surveys for anurans. Each permanent pond was surveyed 3-4 times in 2004, and the temporary ponds 1-3 times, including night observations. The surveys were carried out between March and August. Each pond and its surroundings in a 800 m radius was characterised using a number of habitat variables: 15 variables were used for permanent ponds and 13 for temporary ponds (tables 1-2). The permanent and temporary characters of the ponds were established through repeated surveys effectuated during the whole season (Hartel et al., 2005), the size and vegetation.

The average values regarding species richness and habitat variables between the two areas investigated were compared using Kruskal-Wallis ANOVA by Ranks and Mann-Whitney U test, depending on the differences between the homogeneity of variances (tested using Levene test).

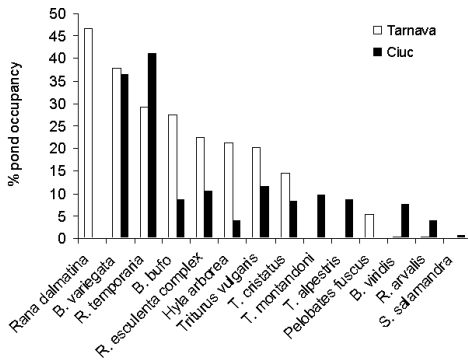
We used hierarchical partitioning (HP) (Chevan and Sutherland, 1991) to evaluate the independent influence of habitat factors on species richness. In HP the goal is to compare the influence of each predicted variable on a response over a hierarchy of all possible  $2^N$  models for  $N$  predictors instead of identifying a single best model (MacNally, 2002). We used the *hier.part* package (Walsh and MacNally, 2004) in the statistical software R version 2.0.1 (R Development Core Team, 2004) to carry out HP for permanent and temporary ponds in both Târnava and Olt valleys.

1 Mircea Eliade College, 545400, Sighișoara, str. 1 Decembrie, nr. 31, Romania. asobeka@yahoo.com

2 Sapientia Hungarian University of Transylvania, Department of Technical and Nature Sciences, 530104 M-Ciuc, pța. Libertății 1, Romania.

3 University of Bucharest, Faculty of Biology, Splaiul Independentei 91-95, 050095 Bucharest, Romania.

4 South Dakota State University, Department of Biology and Microbiology, Brookings, SD, 57007, USA.



**Figure 1.** The percentage of pond occupancy of the amphibian species in the two regions. The data for permanent and temporary ponds are pooled.

Significance of the independent contribution to variance ( $I$ ) for the predictors was assessed using Z-scores, from a comparison of the observed  $I$ s to  $I$ s resulting from 100 permutations on randomized data (MacNally, 2002).

The following habitat variables were considered for permanent ponds (every variable scored as 1 if present and 0 if absent): arable land (the presence of arable land around the pond, coded as present/absent), intensively managed arable land (with chemicals and heavy machineries being used), negligible arable land (traditional land use), road, main road (roads with high traffic volume), main urban areas (combination of buildings, industrial units, roads etc.), and pasture/grass land. For temporary ponds we considered as habitat parameters: main road, dirt road, arable land, intensively managed arable land, negligible arable land and pasture.

|                                     | Târnava               | Ciuc                             |
|-------------------------------------|-----------------------|----------------------------------|
| Elevation (m a.s.l.)***             | 399.2 (278-625)       | 821.18 (638-1301)                |
| Area (m <sup>2</sup> )              | 72156.4 (100-1500000) | 87074.9 (24-810000)              |
| Maximum depth (cm)**                | 233 (30-250)          | 411.18 (6-3800)                  |
| Depth < 30 cm (%)                   | 29.53 (1-100)         | not estimated                    |
| Macrophyte cover (%)*               | 29.25 (1-100)         | 24.6 (0-95)                      |
| pH                                  | 7.56 (6.02-8.94)      | 7.75 (6.14-10.45)                |
| Conductivity (µS/cm)                | 637 (66.2-1828)       | 328 (107.4-683)                  |
| Age (years)                         | 16.9 (2-40)           | 17.5 (1-40, and 5 natural ponds) |
| Distance from forest (m)**          | 231.69 (0-1200)       | 1312.27 (0-3000)                 |
| Green corridor (%)                  | 77.42                 | 54.55                            |
| Arable land (%)                     | 54.84                 | 22.73                            |
| Intensively managed arable land (%) | 6.45                  | 4.55                             |
| Negligible arable land (%)          | 48.39                 | 22.73                            |
| Road (%)                            | 51.61                 | 45.45                            |
| Main road (%)                       | 30.65                 | 13.64                            |
| Main urban areas (%)                | 19.35                 | 18.18                            |
| Pasture/grass land (%)              | 79.03                 | 77.27                            |

**Table 1.** Comparison of the variables describing habitat characteristics of the permanent ponds and their surroundings. Values in parentheses: minimum and maximum. Area, pH, conductivity and the distance from the forest were compared using ANOVA. Elevation, maximum depth, macrophyte cover and age, were compared using Kruskal-Wallis ANOVA by Ranks. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

## Results

A total of 513 ponds were surveyed during this study, of which 84 are permanent (16%). Of these, 248 ponds are situated in Târnava (62 permanent and 186 temporary), and 265 ponds in Ciuc (22 permanent and 243 temporary). Permanent ponds in Târnava are situated at lower altitudes, have a smaller depth, a larger macrophyte cover, and are closer to the forest than permanent ponds in Ciuc. Green corridors are more frequent in Târnava than in Ciuc. Temporary ponds in Târnava are situated at lower altitudes, have smaller areas, and are closer to the forest than temporary ponds in Ciuc (table 2).

Eleven species were found in Târnava and 12 species in Ciuc, including a species complex (*Rana esculenta*). There are differences between the two regions in species pond occupancy patterns. *Rana dalmatina* and *Pelobates fuscus* are absent in Ciuc, while *T. alpestris* and *T. montandoni* are absent in Târnava. In Ciuc hybrids between *T. vulgaris* and *T. montandoni* were also found. *Rana temporaria* is the most common species in Ciuc, while *Bombina variegata* is widely distributed in both areas. *Bufo bufo*, *Hyla arborea*, *R. esculenta* complex, *Triturus vulgaris* and *T. cristatus* have a higher frequency of occurrence in Târnava than in Ciuc, whereas *B. viridis* and *R. arvalis* are more common in Ciuc (fig. 1). In Târnava there are permanent ponds with 6 to 9 amphibian species, the percentage of ponds with 8 species being relatively large. In Ciuc 14% of the permanent ponds are not used by amphibians, and there are no permanent ponds with more than five species. A larger proportion of

|                                | Târnava          | Ciuc                |
|--------------------------------|------------------|---------------------|
| Elevation (m)***               | 528.1 (304-840)  | 758.57 (634-1505.4) |
| Size (m <sup>2</sup> )***      | 34.01 (0.2-504)  | 654.32 (0.35-30000) |
| Depth (cm)***                  | 20.23 (3-150)    | 36.5 (0-150)        |
| pH                             | 6.98 (5.69-8.95) | Not estimated       |
| Conductivity (µS/cm)           | 506 (113-1448)   | Not estimated       |
| Distance forest (m)***         | 111.75 (0-1000)  | 1649.69 (0-4000)    |
| Green corridor (%)             | 90.32            | 29.64               |
| Macrophytes (%)                | Not estimated    | 32.97 (0-100)       |
| Pasture/grassland (%)          | 40.32            | 71.94               |
| Arable land (%)                | 9.14             | 40.71               |
| Dominant arable land (%)       | 8.60             | 10.67               |
| Negligible arable land (%)     | 0.54             | 28.65               |
| Main road (%)                  | 0.54             | 10.28               |
| Dirt road (%)                  | 45.16            | 27.67               |
| Main roads and urban areas (%) | -                | 9.09                |

**Table 2.** Comparison of the variables describing habitat characteristics of the temporary ponds and their surroundings. Values in parentheses: minimum and maximum. All comparisons were made using Kruskal-Wallis ANOVA by Ranks. \*\*\* $P < 0.001$ .

the temporary ponds are not used by amphibians in Târnava than in Ciuc (fig. 2).

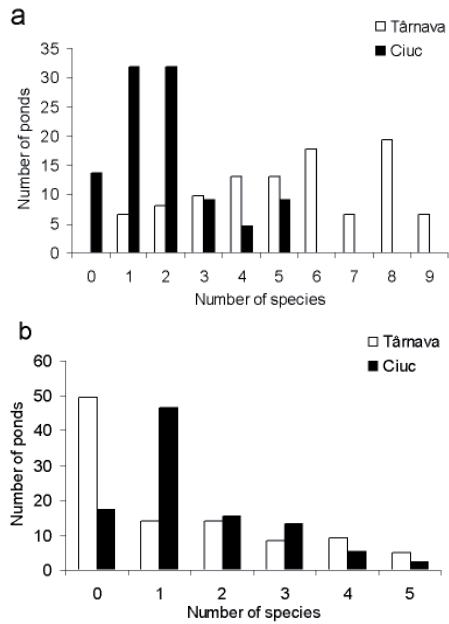
In Târnava, the average species richness is significantly higher in permanent ponds (average 4.66, SD = 3.28) than in temporary ponds (average 1.28, SD = 1.58) (Mann-Whitney U test,  $P < 0.001$ ). No significant differences were found between the species richness in permanent and temporary ponds in Ciuc (average 1.86, SD = 1.42 vs. 1.49, SD = 1.20) (Mann-Whitney U test,  $P = 0.18$ ). The species richness of permanent ponds is higher in Târnava than in Ciuc, whereas temporary ponds have larger species richness in Ciuc than in Târnava (Mann-Whitney U test,  $P < 0.01$  for both pond types).

The hierarchical partitioning analysis indicates that the presence of high traffic roads within an 800 m radius explains more variation in species richness than the other habitat parameters considered in the case of permanent ponds. The presence of dirt roads within a 800 m radius is the most important habitat factor in the case of temporary ponds (fig. 3).

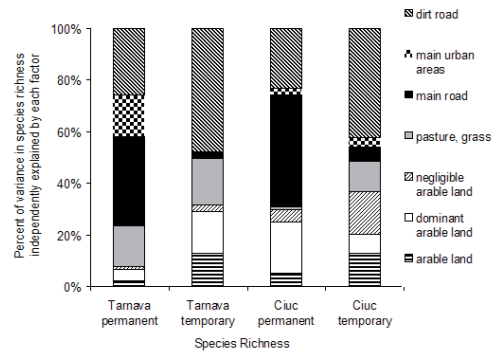
**Discussion**

There are differences between the amphibian species composition in the two areas studied. Thus Târnava is out of the geographical range of *T. alpestris* and *T. montandoni* (Cogălniceanu et al., 2000). The reasons for the absence of *P. fuscus* and *R. dalmatina* from Ciuc was not studied yet. Possible factors may include the colder climate and geographic isolation, and shortage of suitable habitat. This aspect needs further research. The large species number in Ciuc (12) is the result of the topographical heterogeneity of this area which consists of a mountain area with 6 species and a lower altitude area with 8 species.

Species that prefer large permanent ponds for reproduction, such as the *T. cristatus*, *B. bufo*, and *R. esculenta* complex (Laurila, 1998; Cogălniceanu et al., 2000; Babik and Rafinski, 2001) are more common in Târnava, where this habitat type is well represented. Species that prefer temporary ponds for reproduction, such as *B. variegata* (Barandun and Reyer, 1997 a; b) and *B. viridis* (Ghira et al., 2002) occupy a greater percentage of ponds in Ciuc. The significantly larger distance of ponds from the forests in Ciuc combined with a colder climate may be the reason for the very low pond occupancy of *H. arborea* in Ciuc compared with Târnava, as this species prefers forested areas as terrestrial habitat (Fuhn, 1960; Cogălniceanu et al., 2000). In Târnava, the species richness of permanent ponds is higher than that of temporary ponds, most probably due to the higher diversity of microhabitats



**Figure 2.** The distribution of the ponds with different species richness in the two regions: (a) permanent ponds, (b) temporary ponds.



**Figure 3.** Proportion of explained independent variance (relative Is) associated with the anthropogenic factors for species richness in permanent and temporary ponds in Târnava and Olt valleys based on hierarchical variance partitioning modelling.

(larger structural complexity) provided by permanent ponds. In Ciuc, the lower species diversity of both permanent and temporary ponds could be explained by the lower overall species richness within the two altitude zones (6 and 8 species), the relatively low age and low number of permanent ponds. Temporary ponds typical to the low altitude zone of Ciuc have a relatively long hydroperiod (mean 10.4 weeks in 2004, n=48) (Demeter, 2005).

Roads have a huge impact on the environment, with high amphibian mortality caused by traffic being reported in the literature (e.g. Ashley and Robinson, 1996; Lodé, 2000; Smith and Dodd, 2003). Our results indicate that the roads represent the most important source of variation in species richness in the two areas. Similar results were reported in a study of North American salamanders (Porej et al., 2004). Road traffic across the investigated areas will increase in the near future, due to economic development and since many of the local villages are tourist destinations. The negative impact of the roads will also increase with the construction of the motorway, that will cross Târnava at a length of about 15 km. Forman and Deblinger (2000) showed that the significant ecological effects of roads on plants and animals, including amphibians, averages 600 m outward from a road.

In conclusion, the areas investigated still hold a high diversity of habitats and amphibian species. The pond occupancy of individual species shows regional differences. The majority of species in Ciuc have low pond occupancy. Species richness is highest in the permanent ponds from Târnava, whereas there is no significant difference between permanent and temporary ponds in Ciuc regarding species richness. Roads represent the principal source of variation in species richness in both areas.

**Acknowledgements.** We thank to Martha Cowell for improving the English. This study was financially supported by a Declining Amphibian Population Task Force grant (2004).

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