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Local and landscape determinants of breeding pond use by amphibians in central Târnava Mare Valley, Romania

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Abstract

Amphibians are widely recognized as complementary tools for physical planning due to their sensitivity to environmental conditions and strong dependence on breeding, feeding and hibernating habitats, often spatially separated. Starting from this perspective, data on habitat use by the amphibian communities inhabiting the central Târnava Mare Valley in Romania is presented, with the aim to use the findings as management recommendations. A total of 54 permanent ponds were investigated in a 4000 km^2 area in order to identify which landscape characteristics are the most important predictors of the breeding pond occupancy by amphibians. High traffic roads were the most important landscape variables, the pond occupancy of five species was negatively associated with them. Other landscape variables, such as distance from breeding site to forest, presence of green corridor between breeding pond and forest, and different land covers were important only for a small number of species (as much as two species influenced by a variable).
A smaller scale study has been conducted within the Breite woodpasture, a 132 ha protected area threatened by desiccation, affecting several species of conservation interest, and therefore in need of fast management interventions. Eight of the 11 amphibian species from the larger area are present here, but relying solely on temporary water bodies. The only constraint for their reproductive success is the quality of breeding habitats, particularly the hydroperiod. Three aquatic habitat types were considered: temporary ponds, drainage ditches and archaeological ditches. Both species richness and successful metamorphosis were higher in the aquatic habitats with longer hydroperiods, namely the drainage ditches. The maintenance of four species strongly depends on the presence of ponds with long duration. Management interventions are proposed in this direction.

Keywords: amphibians, breeding pond use, conservation, landscape, management, Romania

Contents

Introduction

The need for environmental assessment regarding physical infrastructure development and land-use changes is increasingly recognized, and for several countries it is compulsory by legislation (Löfvenhaft et al. 2004). In order to formulate predictions about the possible effects of land cover changes that follow planning works, scientists are often required to present studies in short time (Nilsson & Grelsson 1995). Even though there are still gaps to be filled in basic data about the biology, habitat use, spatial and temporal distribution patterns of individual species, critical elements needed to formulate conservation recommendations are already known for several groups, amongst them amphibians (Semlitsch 2002).

Amphibians have been long recognized as sensitive indicators of environmental qualities, and in the same time they are in a widespread global decline (Corn 2000; Houlahan et al. 2000; Kiesecker et al. 2001; Stuart et al. 2004). The causes of this decline are diverse, including habitat destruction and alteration (Carrier & Beebee 2003; Dodd & Smith 2003; Ficetola & Bernardi 2004), climate change and its consequences, including shifts in precipitation and temperature regimes (Pounds et al. 1999), increased UV-B radiation (Merila et al. 2000), and increased exposure to parasitic infections (Pounds et al. 1999; Kiesecker et al. 2001), infectious diseases (Berger et al. 1998; Lips 1998; Bosch et al. 2001), acidification, chemical pollutants (Harte and Hoffmann 1989; Mann et al. 2003), and introduced predators, especially fish (Bradford et al. 1993; Brönmark & Edenhamn 1994; Gillespie & Hero 1999; Matthews et al. 2001; Hartel et al. 2007a). Loss of habitat by outright destruction, degradation and fragmentation (Bressi 1998; Anderson et al. 2004) is often cited as the most significant anthropogenic cause of amphibian declines (Corn 2000; Stuart et al. 2004). But habitat loss has many gradations ranging from habitat transformation that is often difficult to recognize such as contamination or the introduction of nonnative species (Kats & Ferrer 2003), road constructions (Vos & Chardon 1998; Puky 2006) to complete destruction of habitats (Dodd and Smith 2003). The complex life cycle, diversity of developmental stages, permeable skin and permanent close contact with the land surface, makes amphibians particularly exposed to environmental perturbations (Lips 1998; Vos & Chardon 1998; Mazerolle & Desrochers 2005). Because their sensitivity and widespread decline, amphibians need fast conservation management interventions, but for the same reasons they fit well the requirement for organisms that can evidence environmental changes in a relatively short time (Petranka et al. 2003) and also enhance conservation interest.

Amphibians, although showing high breeding pond fidelity as adults (Gill 1978), require the proximity and accessibility of suitable wetlands (Nyström 2002) during their dispersal as juveniles (Semlitsch 2000a). Pond-breeding amphibians require both aquatic and terrestrial habitats to complete their life cycles. Even though the habitat types are often spatially separated, they must be co-located in order to provide conditions for the specific requirements of each life stage. As a consequence, the persistence of populations is dependent on the presence and availability of diverse habitats, amphibian diversity increasing with the heterogeneity of landscapes (Anderson & Arruda 2006; Gagné & Fahrig 2007). Sometimes landscape factors are more important than local factors in explaining amphibian species richness (Hecnar & M`Closkey 1998). Having the most complex environmental requirements among amphibians (Dodd & Smith 2003), and a complex connection with landscape structures, pond breeding amphibians offer the opportunity to examine separately the importance of the breeding habitat quality (hydroperiod [Pechmann et al. 1989; Griffiths 1997], habitat complexity [Van Buskirk 2005] and fish introductions [Knapp 2005]) and the role of landscape composition and configuration (Guerry & Hunter 2002).

Amphibians often migrate considerable distances between aquatic and terrestrial habitats (Sinsch 1987, 1990) and require safe corridors for their movements, especially if they must cross human-made structures, such as roads. Besides the major importance of the quality of connections between aquatic and terrestrial habitats (Ray et al. 2002), species maintenance also depends on the distances between ponds, since individuals are restricted in their movements by physiological limitations (Semlitsch 1998).

Knowledge about habitat characteristics is especially important considering that in lack of population demographic data, habitat characteristics represent predictive information on population characteristics and future viability (Scribner et al. 2001). Therefore, when recommendations for land developers have to be formulated in short time, information on habitat characteristics can be regarded as useful impact assessment tool for land use changes (Löfvenhaft et al. 2004).

Romania has many relatively undisturbed ecosystems, and includes the greatest number of biogeographic regions among European countries (European Environmental Agency 2003). Preliminary studies within the Târnava Mare Valley area revealed a rich flora and fauna, with extensive meadows and pastures that are of local, national and international importance (Akeroyd 2000, 2001; Mountford & Akeroyd 2005). As the country's economic development goes ahead, human attitude towards nature will change (Naveh 1998; Foster 2002; Palang et al. 2006), and anthropogenic impact on these diverse habitats will increase. These will likely lead to future decreases in species richness and significant changes in biodiversity. In order to overcome some of these losses, resource managers need baseline data regarding both habitat characteristics and species composition in order to make responsible decisions for nature conservation.

The current study focuses both on landscape studies within a broader area in the central Târnava Mare Valley and on the hydrological changes that threaten the Breite woodpasture, a natural reserve with several-century-old oaks situated in the middle section of the Târnava Mare Valley. Several of the factors that are currently leading to the disappearance of amphibian species at global level can already be observed within central Târnava Mare Valley too. The area is threatened by fragmentation due to a large motorway project that is likely to detrimentally impact regional biodiversity (Blonde & Jacobs 2004, unpublished report).

Both pond and landscape variables were recorded in the preliminary study for Hartel et al. (2007a), but landscape variables were then considered only as binary (present/absent) predictors of breeding pond use by amphibians. The results pointed out that pond (emergent aquatic vegetation cover) and landscape variables (presence/absence of green corridor) are similarly important for amphibian species richness in permanent ponds, followed by roads, predatory fish and non-predatory fish. The individual species occurrence was mainly influenced by pond variables (emergent aquatic vegetation cover, followed by predatory fish and non-predatory fish; results already presented in Hartel et al. 2007a), roads having the greatest effects among landscape variables.

In addition, in the current study I wanted to determine whether or not landscape variables considered as quantitative data would provide additional information to that captured by the categorical parameters (binary presence/absence variables) mentioned before. Ditching on the Breite plateau has lead to an almost complete disappearance of the initial marsh system that characterized the area in the past, jeopardizing the future viability of species of conservation interest. Since a major area within central Târnava Mare Valley has been proposed for Natura 2000 designation, and the Breite woodpasture is already a natural reserve, there is urgent need for management recommendations.

The aims of this study were twofold:

a) To study which pond and landscape parameters are the most important predictors for the permanent pond occupancy of individual species and the amphibian species richness.
b) Within a small scale study, (i) to present the breeding phenology and (ii) evaluate use of temporary aquatic habitat by amphibians, and (iii) to

see into what extent the pond use of individual species predicts for the amphibian species richness and reproductive success and to discuss management implications based on the findings.

Methods

Study area

An area of approximately 4000 km² in the Târnava Mare Valley (central Romania, Fig. 1) was selected for research. The central section of the Valley is dominated by hills ranging in elevation from 750-800 m in the east to 600-800 m in the west, having an important effect on the climate, which is continental. Mean annual temperatures increase from the east to west (from 6.5°C to 9°C). The amount of precipitation ranges from 700-800 mm in the east to 600 mm in the northwest. Dominant land uses consist of approximately 40% pastures, meadows and grasslands, 30% forests (mainly deciduous), 27% agricultural areas; settlements and industrial areas representing around 3%.
A 35 km section the Târnava Mare River was regulated after catastrophic floodings in 1970 and 1975. The canalization of the river and its tributaries resulted in a number of new ponds along the river. On the other hand, the floodplain and the width of the river have been strongly altered. Currently the valley is fragmented by two main roads and constructions of a large motorway that will pass the valley (from northwest to southeast) have already started.

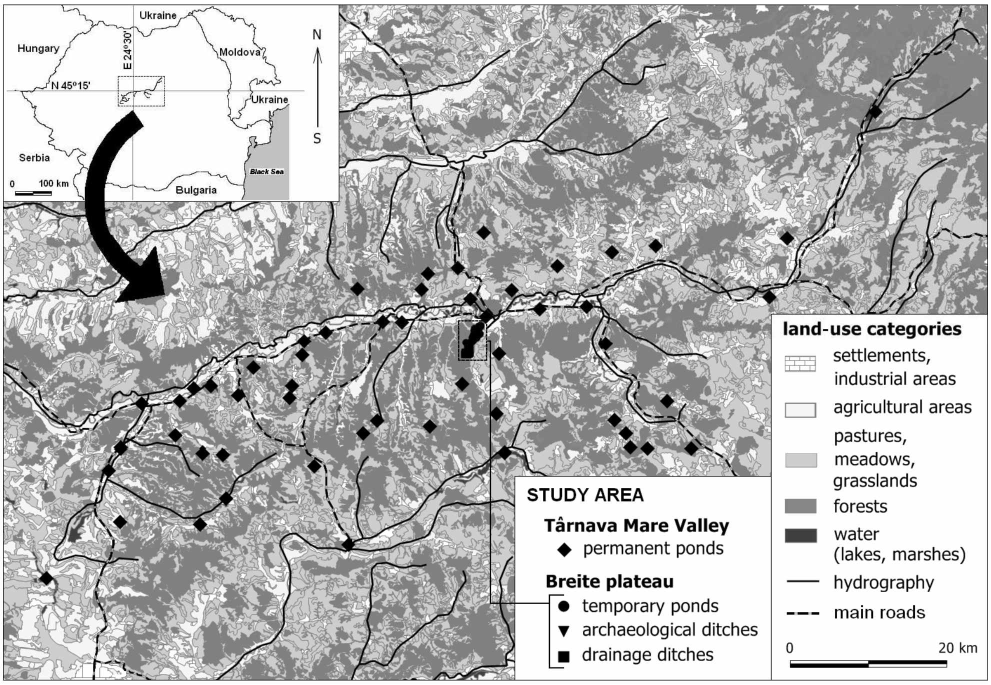


Fig. 1. Map of the study areas. The *Târnava Mare Valley* covers approximately 4000 km^2, while the *Breite plateau* is an area of 132 ha.

The Breite plateau is situated in the middle section of the Târnava Mare Valley, near the town Sighişoara, at about 515 m elevation (Fig. 1). It is a woodpasture, a culturally modified oak forest (Makkai 2003). The area is protected (IUCN IV) due to the presence of 638 scattered several-century-old oaks (Pedunculate Oak *Quercus robur* and Sessile Oak *Q. petraea*, most of the trees being hybrids) (Öllerer et al. unpubl.), being hosts for several species of conservation interest. In the 1980s 15 drainage ditches were built on the plateau with the aim of transforming it into an agricultural area, but no crops were planted at the end. In 2001, 30 archaeological ditches were also dug on the plateau, with the aim to search for artefacts. Besides the dates of their origin, the two ditch categories also differ in sizes, drainage ditches being deeper, wider and longer, therefore retaining water for longer periods. Ditching in the past decades led gradually to an almost complete disappearance of the marsh system that characterized the area in the past (A. Goţa pers. comm.). Today only temporary ponds are available for amphibians breeding in this area. Based on the current knowledge, the amphibian community here is sustained only by breeding, since no immigration from the surrounding ponds has been found.

Amphibian surveys in the Târnava Mare Valley

The study has been conducted on 54 permanent ponds during the 2001-2006 period. All studied amphibian species are pond breeders, laying their eggs in water and having aquatic larvae. Adults, eggs and/or larvae were identified either by dip netting, torch counts during night, or by identifying calling male anurans. Dip netting was carried out for 25 to 60 minutes, depending on the size and accessibility of the site. In order to increase the probability of detecting reproductive adults and larvae in the water, searches were done at least three to four times per season (March-August).

Ponds were located using 1:25 000 scale topographic maps, based on information provided by landowners and by active search. Data on pond altitude and location were obtained using a handheld GPS, accurate to 5 m in average. Land cover data consisted in simplified land-use classes based on data obtained from Coordination of Information on the Environment (CORINE) Land Cover 2000 CLC2000 100m version of Romania (European Environmental Agency 2005), corrected and complemented with on-site observations. Terrestrial habitat characteristics were considered within 400, 600 and 800 m radiuses, centered on the ponds (Lan & Verboom 1990; Joly et al.

2001; Houlahan & Findley 2003). These ranges were selected because they encompass the mean migration distance (400m) of newts (Baker & Halliday 1999; Ray et al. 2002) and an approximate migration distance for some anurans (Reading et al. 1991; Baker & Halliday 1999; Ray et al. 2002). Initially, data for 85 ponds were gathered, removing later from the analysis ponds with overlapping landscapes (Gagné & Fahrig 2007), cases when the 400/600/800m radiuses were sectioning each other, and analyzing landscape variables around 54 ponds at the end. In the case of overlapping landscapes, the ponds with highest species richness were selected for the statistical analysis. Land cover analysis was done with Manifold 7x System Release GIS software (CDA International Ltd. 2006). The measured landscape variables were: elevation (m a.s.l.), roads (present/absent), green corridor (present/absent), distance from breeding pond to forest (m). CORINE land cover data was used in the following classification: settlements (%), agricultural land (%), pasture and grassland (%), forest (%), wet area (%). Vegetation stripes between pond and forest that could act as shelter and were able to provide favorable humidity conditions for amphibian movement, compared to adjacent areas, were considered as green corridors. Roads were considered for low volume traffic ($\leq$ 2 cars / 5 minutes) and high volume traffic ($\geq$ 9 cars / 5 minutes), based on repeated measurements at dusk, when the amphibian migration was the highest.

Amphibian surveys on the Breite plateau

This study was carried out through 2006. A total of 102 temporary water bodies were surveyed, out of which 72 were ponds, 20 were archaeological ditches and 10 were drainage ditches. Water bodies of natural origin, wheel routes and roadside ponds were all considered as "ponds" (Babik & Rafinski 2001). Research begun in the second part of February (after snow melt, when the ponds filled with water) and ended in late September (which is the end of larval period for most species). Amphibian adults, eggs and/or larvae were sampled either by dip netting, torch counts, or by identifying calling male anurans. Dip netting was carried out for 15 to 30 minutes, depending on the size of the site. Intensity of search was increased to at least three times per breeding habitat during the reproductive season of amphibians (early March till end of May). The number of ponds where metamorphosed juveniles were observed was used for estimating reproductive success. Insect predators were also searched for by dip netting (Barandun & Reyer 1997). The areas sampled were adjusted to the size of the individual water bodies, in order to cover 30-40% of each water body. Counts were scored as following: 0 (never observed), 1 (less than 5 individuals or single times), 2 (6-50 individuals) and 3 (> 51 individuals). Each aquatic habitat was marked using handheld GPS, accurate to 5 m in average. Location analyses were done with Manifold 7x System Release

GIS software (CDA International Ltd. 2006). Seven aquatic habitat variables were used to describe the habitats: area (m^2), maximum depth (cm) (measured after snow melt), the percentage of open water (water surface not covered by emergent vegetation, such as the Common Waterplantain *Alisma plantago-aquatica* or the Soft Rush *Juncus effusus*, estimated in mid-May), overall hydroperiod (number of days when each pond holds water, counted from snow melt till the end of September), first hydroperiod (days until the first drying), second hydroperiod (days until the second drying) and the number of desiccations per year. Hydroperiods were assessed by weekly (at least once per week) surveys of the whole plateau. Daily precipitation data were gathered from the Albeşti water station (< 3 km distance) and corrected for personal observations.

Data analysis

Presence/absence data on amphibians in the Târnava Mare Valley for 2001-2006 was pooled for statistical analysis. First step of the analysis consisted in determining the relevant scale (Houlahan & Findlay 2003), by calculating generalised linear models (GLM) with Poisson error distribution (since richness data are count data) for each variable for each of the three scales (400, 600 and 800 m). Each model retrieved then a measure of goodness of fit by using Akaike Information Criterion (AIC). The scale with the lowest AIC value (best fit) was then selected for further analysis, for each variable separately. Species richness was analysed with GLM (Poisson error distribution) and Hierarchical Partitioning (HP) was performed in order to obtain the independent effects of each predictor variable (Chevan & Sutherland 1991, Mac Nally 2002). HP was performed in order to evidence the independent and joint effects of each variable, after testing for multicollinearity ($r < 0.65$). Interactions between different predictors were found. Corridors were correlated with roads ($r = 0.557$, $P = 0.0001$) and distance to forest ($r = -0.652$, p = P.0001). Multicollinearity often occurs when dealing with spatial data (Jesús & Ángel 2004), and in such cases HP analysis is recommended in order to evidence joint effects caused by the linear relationships amongst explanatory variables (R Development Core Team 2006). GLM with binomial error distribution (logistic regression) and also HP were performed for each species. Assessment of model performance was done with area under the receiver operating characteristic (AUC). AUC is a powerful, threshold-independent measure of overall fit that plots sensitivity (true positives vs. false positives) and varies between 0.5 (for a chance performance) to 1.0 for a perfect fit (Fielding & Bell 1997, Jesús & Ángel 2004). Analyses were done using R software (R Development Core Team 2006).

Predictor variables of temporary pond use on the Breite plateau were plotted against species richness and individual species in a Binary Response Model using logistic regression (Long 1997). Non-significant terms were stepwise removed and the final model contained only significant terms. Assessment of model performance was again done with AUC. The comparison of means was made with parametric test (t test, one-way ANOVA and subsequent Fisher LSD post-hoc test), or non parametric test, for medians (Kruskal-Wallis ANOVA and Mann-Whitney U test,). Differences in homogeneity of variances were checked with Levene test. Analyses were performed with the Statistica 6.0. software.

Results

Landscape influences in the Târnava Mare Valley

11 amphibian species were found in the study area: Great Crested Newt *Triturus cristatus,* Common Newt *T. vulgaris,* Common Toad *Bufo bufo,* Green Toad *B. viridis,* Agile Frog *Rana dalmatina,* Common Frog *R. temporaria,* Moor Frog *R. arvalis* (found only in 5 ponds and therefore considered just for the species richness study)*,* Water Frog *R. esculenta* complex*,* Yellow-bellied Toad *Bombina variegata,* Common Spadefoot Toad *Pelobates fuscus,* and the Tree Frog *Hyla arborea* (Fig. 2).

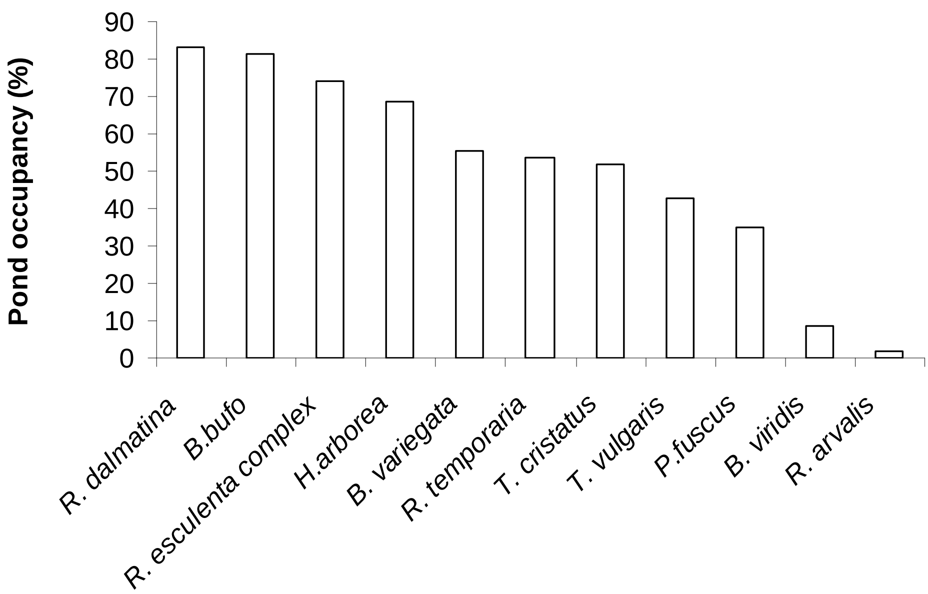


Fig. 2. The pond occupancy of amphibians in the studied water bodies.

Single species analysis

Single species models relating pond occupancy to landscape parameters showed high model accuracy in seven species (AUC ranging from 0.81 to 0.95) excepting *T. cristatus* and *B. variegata* (AUC = 0.67 and 0.64 respectively) (Table 1). High traffic roads were the most important landscape elements that influenced breeding pond use by amphibians in this area. The pond occupancy of five species was negatively associated with the presence of the high traffic roads: *T. vulgaris*, *R. dalmatina*, *R. temporaria, B. bufo* and *H. arborea*. Low traffic roads negatively affect *R. esculenta* complex, *H. arborea* and *B. variegata*. The spatial scale (400, 600 or 800m) at which the negative effect of roads was evidenced differed among individual species (Table 1).

Table 1. Summary of the logistic regression on the relationship between landscape variables and amphibian pond occupancy.

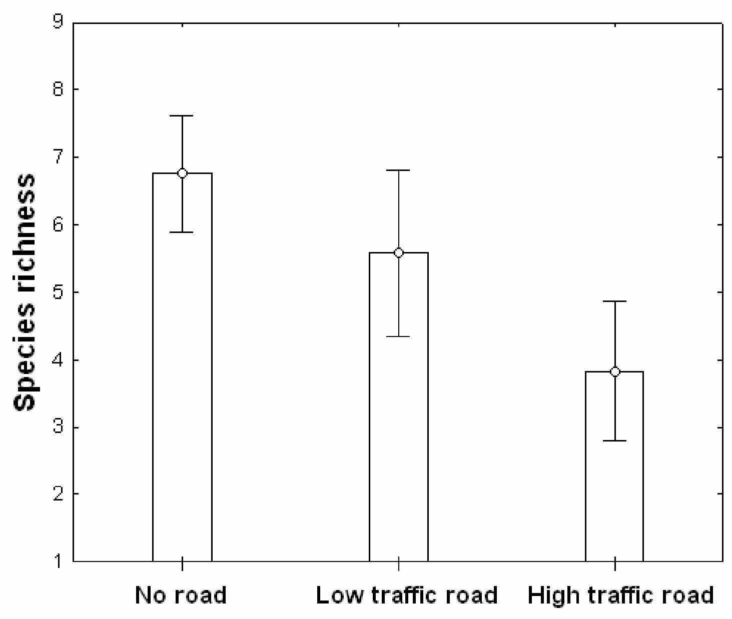
Species	Landscape variables	ß	SE	P	AUC
Triturus cristatus	Forest distance	-0.001	0.0009	0.03	0.67
Triturus vulgaris	H traffic road (400m)	-2.91	1.38	0.03	0.80
Rana dalmatina	H traffic road (400m)	-3.14	1.21	0.009	0.84
Rana temporaria	H traffic road (800m)	-3.37	1.34	0.01	0.95
	Forest (800m)	0.07	0.03	0.03	
Rana esculenta complex	Elevation	-0.02	0.01	0.01	0.90
	Size of other wetlands	-0.28	0.11	0.01	
	L traffic road (400m)	3.96	1.77	0.02	
	Arable lands (400m)	-0.11	0.05	0.04	
	Forest (800 m)	-0.10	0.05	0.04	
Bufo bufo	H traffic road (600m)	-4.14	2.00	0.03	0.85
	Green corridor (400m)	3.73	1.01	<0.001	
Bufo viridis	Pasture (800m)	-0.08	0.04	0.03	0.90
Hyla arborea	L traffic road (600m)	-2.56	0.93	<0.001	0.81
	H traffic road (600m)	-3.15	0.92	<0.001	
Pelobates fuscus	Settlement (400m)	-0.12	0.05	0.01	0.80
	Arable lands (600m)	-0.12	0.05	0.01	
	Wet area (800 m)	-0.16	0.07	0.03	
	Forest distance	-0.015	0.05	0.005	
	Pasture (600m)	-0.15	0.05	0.007	
Bombina variegata	L traffic road (600m)	-1.44	0.73	0.04	0.64

n = 54

There was a significant positive relationship between the low traffic roads within 400 m and the pond occupancy of *R. esculenta* complex. Variables related to forests were significant in the case of four species. The distances of the breeding site from the forest negatively affected the pond occupancy of *T. cristatus* and *P. fuscus*, while amount of forest within 800 m had a positive effect on *R. temporaria*. Presence of green connecting corridor between the pond and the forest is a good predictor for the pond occupancy of *B. bufo* but only at 400m. Joint effects of green corridors and distance to forest were noted in the analyses for four species: *B. bufo, H. arborea*, *R. dalmatina* and *R. temporaria*. Landscape composition was in general of little importance. Other land uses beside amount of forest only affected *B. viridis, P. fuscus* and the *R. esculenta* complex. Both amount of pasture an arable lands had a negative effect on the presence of *P. fuscus*, while arable lands affected negatively the *R. esculenta* complex. Effects of amount of settlements were found only for *P. fuscus* at 400m.

Species richness

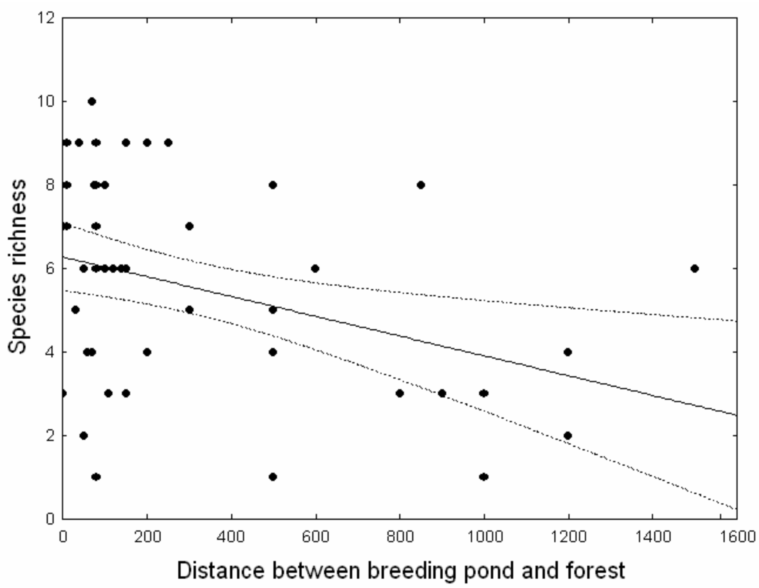
The only significant factor with which the species richness was influenced in the multiple regression is the high volume traffic road (P < 0.001). The species richness is significantly larger in ponds having no roads than in those being closely situated to roads, especially the high volume traffic roads (ANOVA, F = 9.59, P < 0.001, post hoc test, P < 0.05) (Fig. 3).



ANOVA for amphibian species richness. Fisher LSD for post-hoc comparison showed no difference between ponds without roads and ponds with low traffic road (*MS* = 4.54, df = 51, *P* > 0.05). Species richness in both cases (no road and the low traffic road) was significantly larger than for the high traffic roads (both *P* < 0.05).

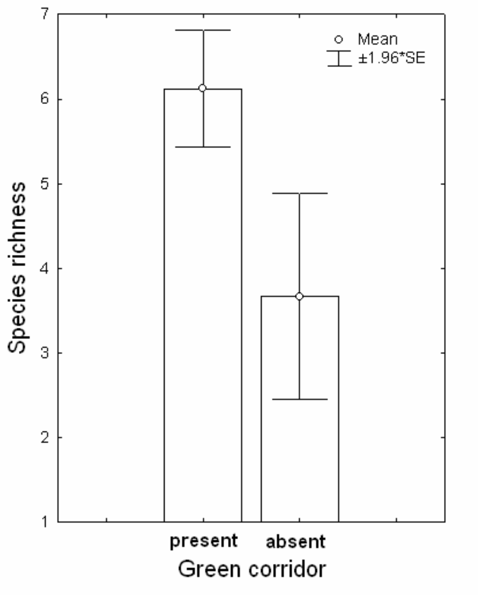
Fig. 3. The species richness in relation to road categories.

The relationship between the species richness and the landscape features was also tested with Pearson correlation. There was a significant negative relationship between the distance from the forest and the species richness (R^2 = 0.13, P < 0.05, Fig. 4). Moreover, ponds connected to a forest by green corridor have higher species richness in average than isolated ones (T-test t = 3.33, df = 52, P < 0.05) (Fig. 5).



Linear regression with 95% CI regression bands ($R^2 = 0.13$, MS = 5.31, df = 52, $P < 0.05$, n = 54)

Fig. 4. The relationship between amphibian species richness and distance between pond and forest.



Levene test (P > 0.05). T-test independent by groups (t = 3.33, df = 52, P < 0.05, n = 54)

Fig. 5. Average species richness in ponds related to presence/absence of green corridors between ponds and forest.

Temporary pond use by amphibians on the Breite Plateau

Eight amphibian species were found in the study area: *Triturus cristatus, T. vulgaris, Bufo bufo, Rana dalmatina, R. temporaria, Bombina variegata, Pelobates fuscus,* and *Hyla arborea.*

Aquatic habitat characteristics

The three habitat types were not equally distributed in the studied area: archaeological ditches are present mainly in the northern part of the plateau whereas the drainage ditches can be found in the central and southern part. The ponds are distributed evenly throughout the studied area (Fig. 6).
There are large differences in the physical parameters describing the three aquatic habitat types studied (Table 2). The most stable habitat types (in terms of hydroperiod and desiccation rate over the season) are the drainage ditches followed by the archaeological ditches and finally the temporary ponds. The length of hydroperiod successfully predicted the type of different ponds ($\beta \pm$ 1SE: 0.044 ± 0.009, $Z = 4.65$, $P < 0.001$).

Fig. 6. The distribution of the three aquatic habitat types on the Breite plateau

Table 2. The physical characteristics of the studied habitat types on the Breite reserve.

Parameter	Habitat type - average (standard deviation)		
	Pond	Archaeological ditch	Drainage ditch
Area (m²)	58.44 (114.32)	21.57 (6.88)	35 (15.80)
Maximum depth (cm)	17.76 (10.34)	29.31 (14.51)	36 (13.29)
Open water surface (%)	48.19 (40.59)	59.47 (27.55)	55 (17.79)
Overall hydroperiod (days)	52.02 (28.06)	62.05 (27.78)	80.5 (33.50)
First hydroperiod (days)	46.5 (23.54)	58.63 (31.27)	79.1 (35.93)
Second hydroperiod (days)	14.68 (11.6)	17.21 (31.82)	26.6 (52.46)
Number of desiccations	6.45 (2.33)	5.3 (2.61)	4.3 (3.02)

n = 102

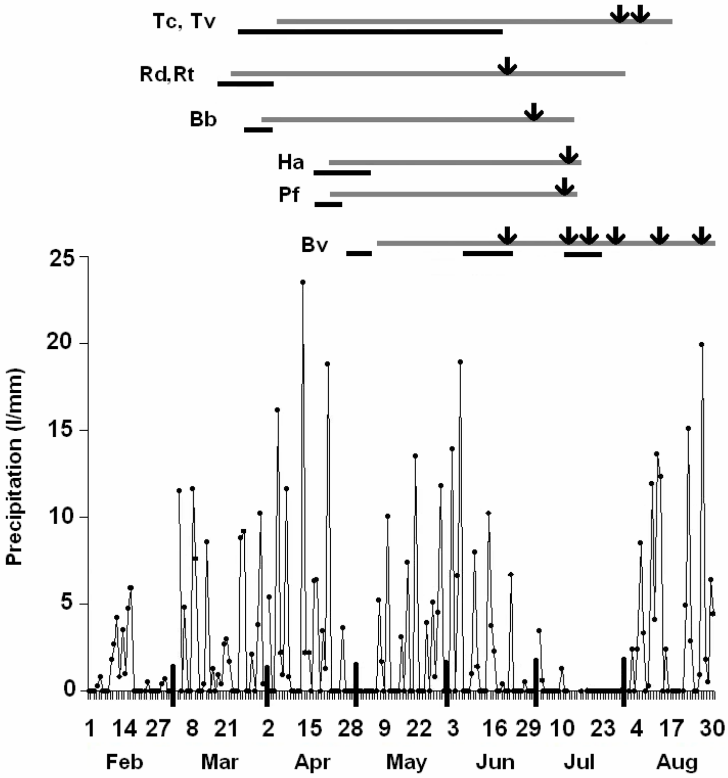
The breeding season and the larval period of amphibians in 2006

The anurans, except *B. variegata* have one egg deposition period lasting for less than two weeks (Fig. 7). No obvious temporal segregation of the start of egg deposition period was observed for *R. dalmatina*, *R. temporaria*, *B. bufo, T. cristatus* and *T. vulgaris*. The egg deposition periods overlap in large part for these species (Fig. 7). *P. fuscus*, *H. arborea* and *B. variegata* start to reproduce after *R. dalmatina*, *R. temporaria* and *B. bufo* have finished the egg deposition. The two newt species, *T. cristatus* and *T. vulgaris,* have a long egg deposition period, lasting until late June. For *B. variegata* three egg deposition events were registered in 2006, the first two being triggered by the increased amount of precipitation and occurred in rainy seasons, whereas the last one occurred during the dry season (Fig. 7). In this case the reproduction was restricted to the habitats with the longest hydroperiod (drainage ditches and archaeological ditches).The model with all explanatory variables included evidenced only three significances: the overall hydroperiod, the length of first hydroperiod and the number of egg deposition events (Table 3).

Table 3. The results of logistic regression on the relationship between larval density categories and explanatory variables.

	ß	SE	z	P	95% CI	
Habitat type	-0.215	0.147	-1.46	0.144	-0.504	0.073
Odonata larvae	0.109	0.118	0.92	0.357	-0.122	0.340
Area	0.000	0.001	0.33	0.739	-0.001	0.002
Depth	-0.004	0.010	-0.41	0.683	-0.025	0.016
Open water surface	0.002	0.003	0.56	0.579	-0.005	0.009
Overall hydroperiod (days)	-0.124	0.057	-2.18	0.029	-0.236	-0.012
First hydroperiod (days)	0.128	0.053	2.43	0.015	0.024	0.233
Egg deposition events	0.894	0.175	5.09	0.000	0.550	1.238
Adult counts	0.004	0.002	1.93	0.053	-0.000	0.009
Constant	-0.199	0.767	-0.26	0.795	-1.704	1.30

n =102

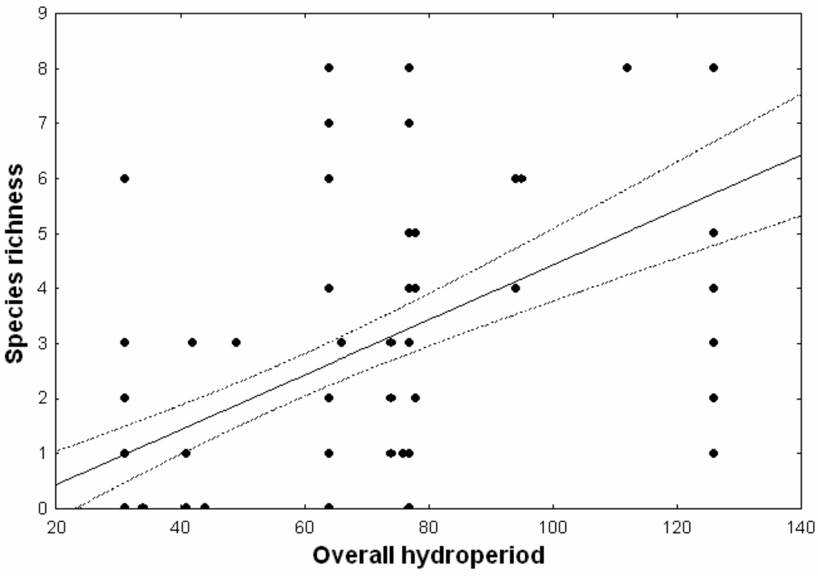


Egg deposition periods (black lines), presence of amphibian larvae (grey line). The precipitation is shown for the active season of amphibians. Tc = *Triturus cristatus*, Tv = *T. vulgaris*, Bb = *Bufo bufo*, Rd = *R. dalmatina*, Rt = *Rana temporaria*, Ha = *Hyla* arborea, Pf = *Pelobates fuscus*, Bv = *Bombina variegata*. The arrows represent periods of intense metamorphosis.

Fig. 7. The egg deposition periods and the presence of amphibian larvae throughout the season on the Breite plateau.

Single species and community-level reproductive success

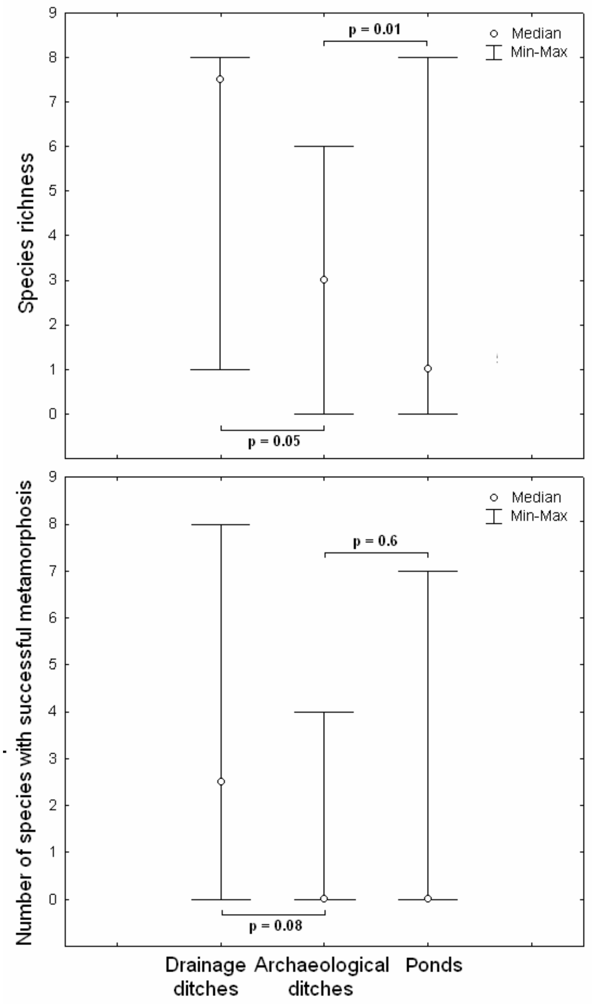
There was a positive relationship between the pond hydroperiod and the species richness ($R^2 = 0.37$, $P < 0.05$, Fig. 8). The reproductive success was the highest in the drainage ditches. Both the amphibian species richness and the number of species that successfully metamorphosed are significantly different in the three aquatic habitat types (Kruskal-Wallis test, $P < 0.05$ in both cases), being higher in the drainage ditches than in the archaeological ditches and ponds (Mann-Whitney U test, $P < 0.01$). There are no significant differences between the ponds and archaeological ditches regarding the number of species with successful metamorphosis (median = 0 [min = 0, max = 7] for ponds and 0 [0, 4] for archaeological ditches) (Mann-Whitney U Test, $P > 0.05$, $n = 72$) (Fig. 9).



GLM for overall hydroperiod (number of days when pond/ditches held water) and species richness) with 95% CI regression bands ($R^2 = 0.37$, MS = 3.84, df = 100, $P < 0.05$, n = 102). Spearman by rank correlation between hydroperiod and species richness (r = 0.65, $P<0.05$)

Fig. 8. The relationship between pond hydroperiod and amphibian species richness

Nearly all species have the largest aquatic habitat occupancy and metamorphosis rate in the drainage ditches (Fig. 10). The reproductive success of *B. variegata* is higher than that of other species in the ponds and archaeological ditches. This species is followed by *R. temporaria*. Two species, *R. dalmatina* and *R. temporaria* metamorphosed in a larger percentage from the drainage ditches than *B. variegata* did.



Kruskal-Wallis ANOVA for species richness (H (2, n = 102) =16.36422, P < 0.001) and successful metamorphosis (H (2, n = 102) =7.641214 P < 0.05). p values are the results of the Mann-Whitney U test.

Fig. 9. Average species richness and average number of metamorphosing species in the three aquatic habitat types in 2006.

Evaluating the predictor value of individual species for occurrence and metamorphosis at community level

The presence of species such are *T. cristatus*, *H. arborea* and especially *B. bufo* and *P. fuscus* indicate high amphibian species richness (up to other seven species present, from the eight identified) and metamorphosis (Table 4). *B. variegata* is a poor species richness and reproductive success predictor, followed by *R. temporaria*.

Moreover, the metamorphosis of *T. cristatus*, *B. bufo, H. arborea,* and *P. fuscus* is a good predictor for the metamorphosis of other species (Fig. 11). In this case too, *B. variegata* and *R. temporaria* are poor indicators of reproductive success at the community level (Fig. 11).

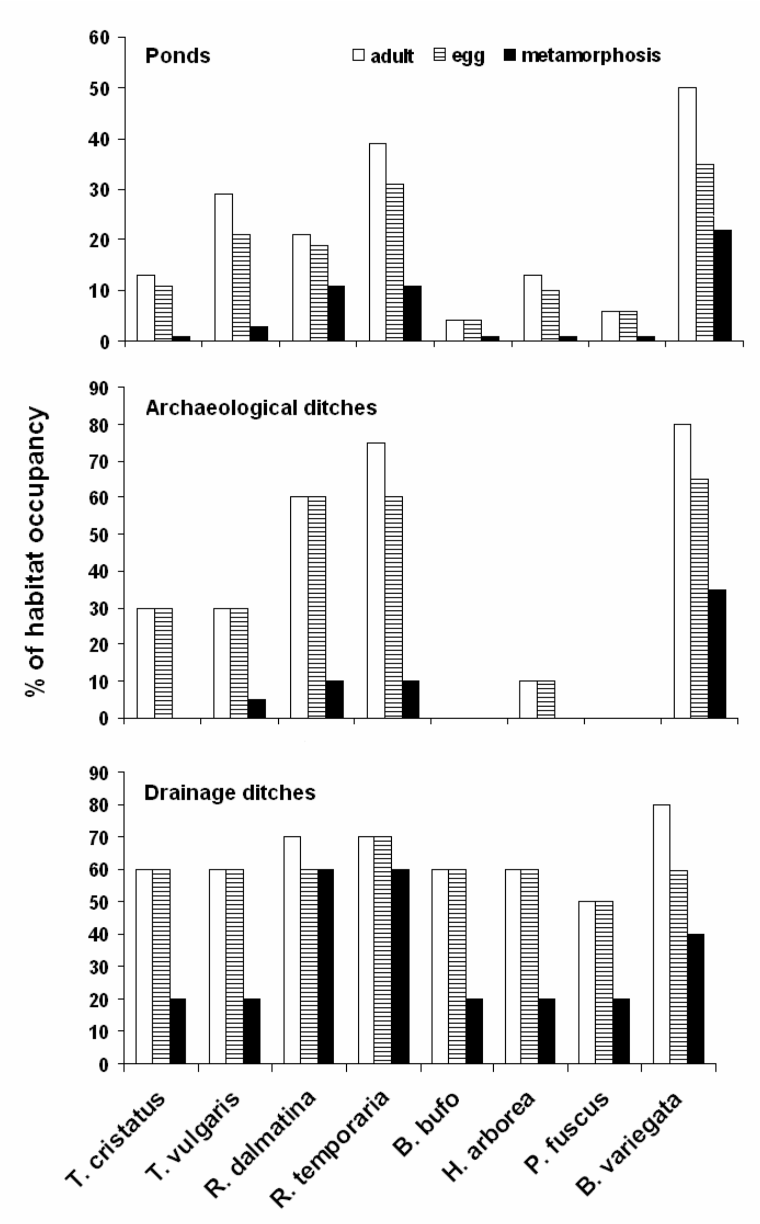


Fig. 10. The presence of different life stages (adults, eggs and metamorphs) in the three aquatic habitat types in 2006

Table 4. Using occurrence data on individual amphibian species as predictors of amphibian species richness and number of metamorphosing species for a given breeding pond (Mann-Whitney U test).

Species	Present/ Absent	Species richness median (SD)	Nr. of metamorphosing species - median (SD)
Triturus cristatus	*P*	5 (1.39)***	3 (2.34)***
	A	1 (1.38)	0 (0.69)
Triturus vulgaris	*P*	4 (1.92) ***	1 (2.17) ***
	A	1 (1.10)	0 (0.43)
Rana dalmatina	*P*	4 (1.90) ***	1 (2.19) ***
	A	1 (1.04)	0 (0.30)
Rana temporaria	*P*	3 (2.12) ***	1 (2.01) ***
	A	0 (0.56)	0 (0.23)
Bufo bufo	*P*	7 (3.04)**	3 (3.15)**
	A	1 (2.05)	0 (0.96)
Hyla arborea	*P*	6 (1.26) ***	3 (2.44) ***
	A	1 (1.54)	0 (0.81)
Pelobates fuscus	*P*	7 (0.50) ***	3 (2.35) ***
	A	1 (1.91)	0 (0.86)
Bombina variegata	*P*	2 (2.31) ***	1 (1.88) ***
	A	0 (0.53)	0 (0)

n = 102
** $P < 0.001$
*** $P < 0.0001$

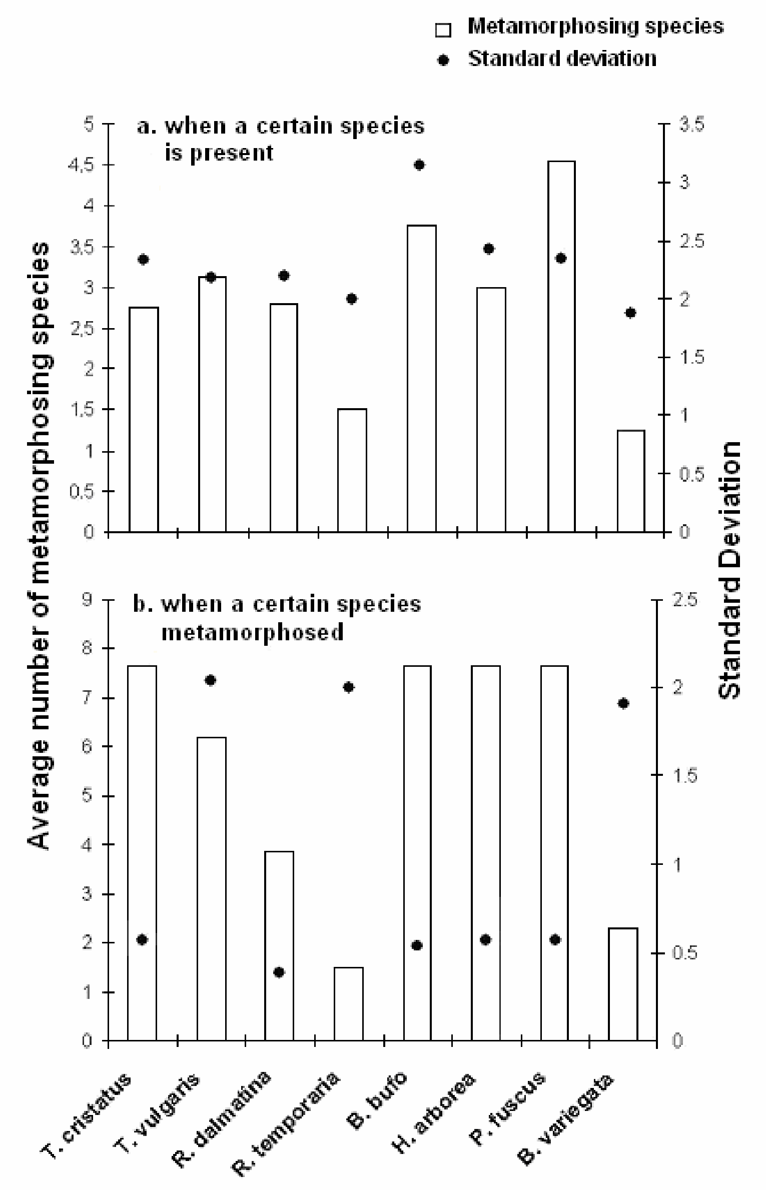


Fig. 11. The average number of metamorphosing species in relation to presence and metamorphosis of individual species

Discussion

Landscape determinants of breeding pond use

A finer species- and land-use specific data used in this study allowed a more detailed approach of landscape influences breeding pond use by amphibians, compared to the previous binary presence/absence records of landscape variables. Results from this study differed for the three spatial scales considered and for the different road categories, pointing out the importance of addressing management issues in a detail and at a scale that is most relevant for individual species and species richness (Johnson et al. 2001; Houlahan & Findlay 2003).

High volume traffic roads represent the landscape element with the largest impact, negatively affecting five amphibian species. Moreover, the species richness was lower in the case of ponds situated nearby high traffic roads than for ponds having low traffic roads or no roads in their vicinity. Different species varied in their sensitivity to different road categories. *Hyla arborea,* for example, shows sensitivity both for high traffic and low traffic roads. Roads have a huge impact on the environment, affecting also the distribution of amphibians (Lodé 2000; Hels & Buchwald 2001; Lesbarreres et al. 2003; Smith & Dodd 2003). Roads modify the permeability of the habitat matrix and change the movement behavior of amphibians, thus hampering the dispersal and migration of juveniles and adults (Ray et al., 2002). Recent surveys made along the Târnava Mare Valley revealed massive road mortalities in the case of *Rana dalmatina*, *R. temporaria*, *H. arborea* and *Bufo bufo* during their breeding migration (Hartel et al. *unpublished data*.). No migration was evidenced for *Triturus cristatus, T. vulgaris* and *Pelobates fuscus* across the roads. However, adults and larvae of *T. cristatus* and *P. fuscus* were detected in ponds close to major roads (Hartel et al. unpublished). The results suggest that these particular amphibian populations might be "trapped" in some ponds along the road and survive as "patchy populations".

Road developments will increase traffic, especially since more and more villages in the area are becoming, or further developing as tourist destinations. Also time lags have to be taken into account, since ecosystems may response only after a certain time has elapsed after following road constructions (Findlay & Bourdages 2000). Considering these, and the fact that the area supports large amphibian communities, communication with land owners and land developers is of major importance for their maintenance (Norton 2000). Awareness is especially important during the breeding season, and as shown for several cases (Puky 2006), strategy-based information of the general public, education and road signs can induce attitudinal changes, therefore contributing to amphibian conservation.

Landscape variables, other than roads, proved to have also an important effect on amphibians. The distance of breeding habitats from the forest was an important determinant of amphibian species richness, showed by the negative correlation between species richness and forest distance. Joint effects of green corridors and distance to forest were noted in the analyses for four species: *B. bufo, H. arborea*, *R. dalmatina* and *R. temporaria,* suggesting that they need both corridors and forests, and that large distances to the forest can be counterweighted by corridors. The proximity and amount of forests positively affects many amphibian populations by ensuring that conditions for feeding, moisture, shelter and hibernation are available for all terrestrial life stages. Because of this, amphibians may be present in high population densities in- and close to forests (Laan & Verboom 1990; De Maynadier & Hunter 1995; Latham et al. 1996; Hecnar & M`Closkey 1998; Joly et al. 2001; Mazerolle et al 2005). Considering that the majority of ponds are situated close to them, forests play an important role in maintaining individual species (Hartel et al. 2007b) and high species diversity in the central Târnava Mare Valley. This conclusion is also supported by the larger amphibian species richness in the breeding ponds with green connecting corridors. Green corridors are especially important for good dispersers, such is *B. bufo*. The results of the current study confirm those of Hartel & Demeter (2005) for this species, studied in one pond that is included in this study also. Hartel & Demeter (2005) showed that a large majority of *B. bufo* individuals migrated from the direction of the forest and through a grassland patch, whereas only a small number of individuals migrated across chemically-treated arable lands. However, not all species can orient (Mazeorlle & Desrochers 2005), coping that way with the loss of land-use types that are suitable for their movements. Joly et al. (2001) found no evidence that linear corridors were a viable substitute solution for the movement of newts in impermeable fields.

Other land-use types (arable lands, pastures, settlements) are also important in explaining the breeding pond use by some amphibian species in the area. The smaller significance of these land-use types, compared to that of the forest, can be explained by the traditional tillage methods that characterize the major part of the study area.

Temporary aquatic habitat use on the Breite plateau

The major difference between the larger study area and the Breite plateau is that in the latter one there is just one major impediment that might hamper the breeding pond use of amphibians, the availability of water. Therefore, the same species (eight out of eleven) that are present in the larger area have to cope with very different environmental conditions on the plateau. *R. esculenta* complex is not present in this area because it would be restricted to use only temporary ponds. Moreover as neither subadults nor adults were identified during the years (Hartel et al. 2005) it can be concluded that no colonization events occur in the area for this species. Breeding in temporary ponds demands different abilities than those necessary in permanent ponds. Flexibility in developmental rate (according to fluctuations in water availability and constrains represented by these fluctuations, particularly the decrease in water level), increased capacity to cope with intra- and interspecific competition is necessary (Schneider & Frost 1996; Welborn et al. 2006).

All ponds and ditches on the Breite plateau are filled with water depending on precipitations. Therefore, the main exogenous environmental factor that triggers the start of breeding activity of amphibians is the air temperature and the amount of precipitation. *Rana dalmatina* and *R. temporaria* are the first that begin spawning, in mid-March. Spawning synchronization with rainfall is especially valid for *Bombina variegata*, species that reproduces several times in favourable seasons (Barandun et al. 1997; Barandun & Reyer 1998), and for which two of the three egg deposition events appeared after precipitation peaks in 2006. Early breeders (early till mid-March) have longer larval stages (Griffith 1997) than late breeders (early till mid-April). In the case of *Triturus cristatus* and *T. vulgaris* the breeding season is also prolonged. The early breeding of these species may have an adaptive role, offering therefore longer time for larval development and increasing the chances of metamorphosis (Pechmann et al. 1989) before the pond eventually dries up (Griffiths 1997). Early breeding is also an adaptation for synchronizing the larval period with the prey availability, resources available per capita being reduced in situations of crowding (Barandun & Reyer 1998; Loman 1999). Late breeding can be advantageous for some species, especially because of the decreased chances of desiccation, compared to the early season, and increased habitat heterogeneity (vegetation), that results in an increase of food resources and possibilities for shelter against predators (Segev & Blaustein 2007).

Three anuran species, *H. arborea*, *P. fuscus* and *B. variegata* begin spawning in mid-April, and for them larvae of early breeding species may be competitors (Griffiths 1997; Segev & Blaustein 2007). Two of these species (*H. arborea* and *P. fuscus*) use mainly the most constant aquatic habitats, namely the drainage ditches, habitats where their highest reproductive success was observed. As the drainage ditches have the longest hydroperiod (some of them holding water through the whole active season of amphibians), the crowding, that may be detrimental for these species in quickly drying pond, is avoided. Since pond desiccation frequently causes catastrophic mortality of larvae (Laurila & Kujasalo 1999), these amphibians have chosen the most stable habitats for reproduction. Species richness increased with pond duration, and therefore the drainage ditches proved to be the most important habitats for overall species richness. Also the drainage ditches were those habitats in which, if the adults were present, egg deposition occurred in all species (Fig. 10).

All species previously observed on the plateau were found also in 2006; however, the reproductive success of the majority of species was low during the last four years. *T. cristatus*, *P. fuscus* and *H. arborea* were not detected until 2005 (Hartel et al.2005). *Bombina variegata*, a typical temporary ponds breeder (Barandun & Reyer 1998), had the highest pond occupancy (both in Hartel et al. 2005 and in this study). *Rana dalmatina* and *R. temporaria* had also successful reproduction in a larger number of ponds, if compared to other species. The rarest species on the plateau are: *T. cristatus*, *H. arborea* and *P. fuscus*. *Triturus vulgaris*, *R. dalmatina*, *R. temporaria* and *B. variegata* are known as species that breed frequently in small water bodies with unpredictable hydroperiod (Barandun & Reyer 1997; Griffiths 1997; Cogălniceanu 1999; Laurila & Kujasalo 1999). These species displayed high plasticity in habitat use (Fig. 10), reproductive adults tended to exploit a larger percentage of the available water bodies, whereas *T. cristatus*, *B. bufo*, *H. arborea* and *P. fuscus* were restricted almost entirely to the most stable drainage ditches. These four latter species were also the ones that predicted best the number of present and metamorphosing species in the individual water bodies.

Having a highly variable reproductive success, the long term maintenance of the amphibian community on the Breite plateau depends mainly on the hydroperiod (ultimately influenced by climate), but also on the recolonization events from potential larger, selfsustainable populations (demographic factor). There is only one permanent pond in the surrounding forest, situated at about 1 500 m distance, used by four amphibian species (notably those four that are most successful breeders on the plateau): *T. vulgaris*, *B. variegata*, *R. temporaria* and *R. dalmatina*. From this pond recolonization may happen for these species, but has not been observed. *Bombina variegata* evidenced high plasticity in habitat use (Fig. 10) and was present in ponds where no other amphibian species were found. Based on its high conservation status (Habitats Directive 92/43/CCE, Annex II), my assumption (rejected by the findings of this study, Table 4) was that *B. variegata* will prove as a good predictor of overall species richness.

Conclusions and management recommendations

Data presented here suggests that decreased species richness is related to decreased landscape connectivity (the pond to forest connection in this case). Landscape connectivity is expected to be gradually reduced, as many of the ponds and their surroundings will be used for recreation, infrastructure and urban development. Such habitat loss has already been observed in 2005 and 2006 for a number of ponds, and development plans already exist for additional ponds and their surroundings. In order to be more efficient, these plans should include cost-effectiveness valuations and alternatives that consider the minimal ecological needs of species, i.e. the extent of habitat heterogeneity that amphibians require to complete their life cycles. It is therefore important to stress out, and communicate land developers and owners (Carr & Hazell 2006), that beside pond characteristics adjacent land-uses (such as roads, forest distances, green corridors, as shown in this study too) also affect amphibian species richness and community composition (Houlahan & Findlay 2003; Mazerolle et al. 2005). Efficient management relies on a simultaneous approach of both pond and landscape scale (Mazerolle & Villard 1999) and also on the identification of the characteristics that favour a maximum number of individuals and species (Bosch et al. 2004). Therefore, an important further step in amphibian conservation in the central Târnava Mare Valley is land developer awareness on the importance of maintaining conditions that assure the long-term persistence of the amphibian communities. This could be realized by formulation of intervention alternatives as a result of close collaboration between landowners, as connoisseurs of local context (Carr & Hazell 2006), planners who have the knowledge of development priorities and scientists that hold information on the specific requirements of amphibians.

Although a protected area at the present, the Breite plateau still suffers the consequences of previous management interventions that aimed intensive agricultural use, rather than maintenance of the traditional woodpasture. The importance of the variety of these water bodies with different hydroperiods is of major importance in maintaining regional amphibian diversity (Semlitsch & Bodie 1998; Semlitsch 2000b). Because of their anthropogenic origin, one would be tempted to fill in the drainage ditches on the Breite plateau in order to recreate the original hydrological conditions and assure the maintenance of species of conservation interest. However, as the present study shows, these ditches represent the most stable habitats for the amphibian community. Therefore in order to secure their long term persistence in this area, ditch enclosure (at the end closest to the plateau edge) is recommended instead of filling up.

The fact that *B. variegata* was not a good predictor of overall species richness and success of metamorphosis lead to the conclusion that it cannot be used as focal species when formulating management recommendations regarding the whole amphibian community. *Bombina variegata* uses short- and long-hydroperod habitats with the same success, whereas most of the species present on the plateau evidenced reproductive success only in the long-hydroperod habitats, namely the drainage ditches. Increased hydroperiod is especially important for those four species (*T. cristatus*, *B. bufo*, *P. fuscus* and *H. arborea*) for which the permanent pond situated in the forest cannot be a potential immigration source.

Therefore, considering that, according to the present knowledge, the amphibian community is sustained only by breeding on the Breite plateau, ensuring a diversity of ponds with varying hydroperiods and creation of ponds with longer hydroperiods is recommended as management intervention for the maintenance of these species.

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