Pond and landscape characteristics — which is more important for common toads (*Bufo bufo*)? A case study from central Romania

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Abstract. The primary anthropogenic factor causing amphibian declines in Europe is habitat loss and fragmentation. Here we explore the effects of aquatic and landscape habitat variables on the adult counts of the Common Toad (*Bufo bufo*) in 43 ponds in central Romania surveyed between 2000 and 2005. Principal components analysis (PCA) identified two main factors, with which the adult counts can be related the first related to landscape variables (percentage of forest cover, presence/absence of roads and habitat corridors) and the second, to pond variables, in particular the presence/absence of fish, together accounting for 49% of the total variance. Analysis of variance (ANOVA) showed differences between ponds where toads were present or absent in factor loadings of both principal components, but only the landscape factor was significantly correlated with toad counts. These results highlight the role of landscape composition and configuration in maintaining toad populations in this area, and suggest a negative effect of landscape fragmentation. To efficiently protect amphibians in Romania, appropriate legislation and a close collaboration between landowners, landscape planners and herpetologists are needed.

Key words: *Bufo bufo*; counts; conservation; habitat features; Romania.

Introduction

The primary anthropogenic cause of amphibian decline in Europe is loss of habitat by destruction and fragmentation (Stuart et al., 2004). Pond breeding amphibians are particularly sensitive to habitat loss and fragmentation due to the spatial heterogeneity of the habitats that they require to complete their life histories and due to their complex life cycles (Wilbur, 1980). The complexity of amphibian habitat requirements results, in part, from the fact that reproductive and terrestrial habitats are often spatially separated, yet must be co-located in order to provide adequate conditions for the specific requirements of each life stage. Amphibians often migrate considerable distances between aquatic and terrestrial habitats (Sinsch, 1987, 1990) and require safe corridors for migration, especially if they must cross human-made structures, such as roads, that may cause severe mortality (Hels and Buchwald, 2001).

The common toad (*Bufo bufo*) is widely distributed in Europe (Gasc et al., 1997). It spends most of the year in terrestrial habitats, where it establishes a home range to which it is phylopatric (Sinsch, 1987). The aquatic phase of the adults is restricted to a short breeding season (they are explosive breeders, sensu Wells, 1977). The adults of reproductive age show high breeding site fidelity. In any year 79-96% of surviving adults return to the original pond (Reading et al., 1991). Several aspects of the spatio-temporal dynamics of populations of this species have been relatively well studied in Western Europe, including: juvenile dispersal from the natal pond (Reading et al., 1991), seasonal aspects of migratory behaviour, home range and breeding pond fidelity (e.g. Sinsch, 1987, 1988, 1990; Reading et al., 1991) and long term fluctuations in population size (e.g. Passenheim et al., 2001). Recent field studies and the development of spatial models have shown that the population size of common toads is positively influenced by some critical habitat elements such as pond density and quality (Halley et al., 1996), presence of woodland, hedgerows, pastures (Scribner et al., 1997, 2001; Sztatecsny and Schabetsberger, 2005) and the quality of the habitat matrix where the migration occurs (Ray et al., 2002; Joly et al., 2003). The common toad is in decline in lowland England but the factors causing this decline are unclear (Carrier and Beebee, 2003). This species has also been reported to be in decline in southern Iberia (Lizana, 2002).

The relatively low level of rural development in Romania during the 45 year communist era has left intact many ecosystems that presumably still hold a high level of biodiversity and large populations of species that are in decline in other areas of Europe. To implement efficient conservation measures at a regional level, baseline data are needed regarding local distributions and the factors influencing habitat use of different amphibian species. To date there have not been such studies in Romania, although many faunal records exist (Hartel, 2005). Such data could be used to assess changes in pond occupancy, population size and community structure at local and regional levels, and factors that may be responsible (Martinez-Solano et al., 2003; Crochet et al., 2004).

The goals of the present paper are:

(i) to characterize breeding ponds and terrestrial habitats of the common toad (*B. bufo*) in central Romania,
(ii) to identify the most important factors influencing the counts of the Common Toad in the study area.

Methods

Study area

The Târnava Mare Valley is located in central Romania. An area of approximately 2600 km^2 was selected for this study in the central section of the valley (fig. 1). This section is dominated by hills ranging in elevation from 600 to 800 m in the west to 750-800 m in the east. A 35 km section of the Târnava Mare River was regulated by control agencies after damaging floods in 1970 and 1975. During the same period, reservoirs were constructed. Regulation of the river and its tributaries during the last 40 years resulted in the creation of a number of ponds along the river, by cutting off the old meanders. Important land use types in the area include: deciduous woodland (33%), shrub (5%), pastures and grassland (41%), orchard (2%), vineyard (1%), marsh (1%) and urban area (1%). Two large roads (fig. 1) and one railway run through the valley. Another highway is planned to be constructed through this valley in the near future (fig. 1).

Data collection

Ponds were located using 1:25 000 scale topographic maps based on information provided by landowners and through searches. The altitude and the exact location of the ponds were established using a handheld GPS. The surveys were made between

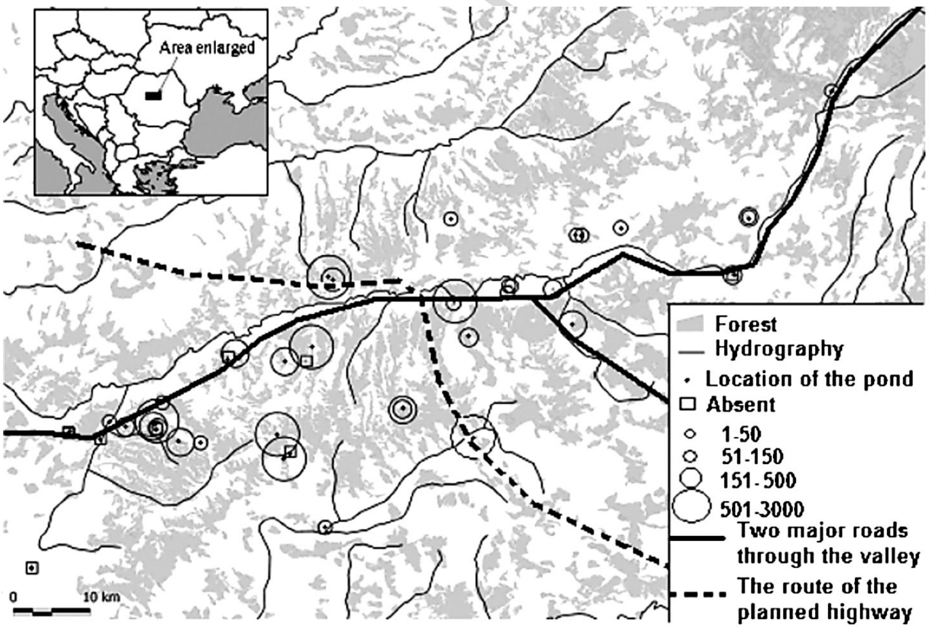

Figure 1. The distribution of the ponds and the common toad counts in the Târnava Mare Valley in central Romania (inset), showing main rivers, forest cover and toad abundance at each site. Parts of national and international roads and the route of the planned highway are also shown.

2000 and 2005. Adult toad counts were gathered for 43 populations in the afternoon and at night (18:00-24:00 hours) from the end of March until the second half of April. Toads were counted during the breeding period along the shoreline (Scribner et al., 2001; Hartel, 2004). Toads in the water or within a perimeter of about 3 m of the land around the pond were counted. When multiple year surveys were made in one pond, the highest count numbers were used as an estimate of population size. At least two visits were made to each pond during the breeding season. Field observations (Hartel and Demeter, 2005; Hartel, unpublished data) allowed preliminary identification of peak activity periods for toad populations, which did not differ significantly in timing among sampling.

We measured four aquatic habitat and seven landscape variables at each sampled site. The aquatic variables were: size (m^2), percentage of emergent aquatic vegetation cover (*Phragmites* sp., and *Typha* sp.), percentage of shallow water (<50 cm depth) and presence/absence of non-predatory and assumed predatory fish. The estimations of the percentage of water shallowness were based on measurements of the water depth (in cm) at different points along the shoreline in the toad breeding season. In the statistical analysis we used the values of the estimated percentage of shallow water from the year in which the last toad population count was made in a particular pond. The presence of fish was determined using visual counts, dip netting and information gathered from fishermen and pond owners. The fish species were grouped into two categories: non-predatory fish (*Carassius auratus*, *Cyprinus carpio*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Leucaspius delineatus*, *Scardinius erythrophthalmus*), and predatory fish (*Esox lucius*, *Squalius cephalus*, *Lepomis gibbosus*, *Perca fluviatilis*, *Pseudorasbora parva*, *Silurus glanis*, *Stizosteidon lucioperca*, *Salmo fario*). A detailed presentation of the different methods used to detect the presence of fish, and also the criteria on the base of which the different fish species were categorized as predators or non — predators are presented in detail in Hartel et al. (2007). The percentage of the emergent vegetation cover was estimated according to Hartel et al. (2007).

Landscape characteristics were recorded in an approximately 800 m radius of the ponds. This distance encompasses the approximate migration distance for *B. bufo* (Reading et al., 1991; Sinsch, 1988). These variables included: distance of pond from forest (m), the percentage of forest cover, the presence/absence of green connecting corridors percentage of pastures and grassland cover, percentage of arable land cover, and the presence-absence of high traffic roads (table 1). Elevation (m) of the pond was also considered as a landscape variable (table 1). The green connecting corridor is a variable that shows the connectivity of the breeding ponds with the forest. We define as green corridors those landscape elements that allow toads a safe migration between the breeding habitat and the forest. Our personal observations show that such landscape elements can be meadows, pastures, small brooks and hedgerows that are situated between the pond and forest. When no such connection was present between the pond and the forest the green connecting corridor was considered to be absent. The green connecting corridor is a variable

Table 1. Definitions and units of measurement for all aquatic and landscape variables recorded. The methods used to estimate each variable are presented in the Methods section.

Variables	Definition of variables/units of measurement
Aquatic variables	
Area	m^2
Shallow water	% of water depth < 50 cm
Emergent aquatic vegetation cover	% over total pond area
Fish	0 = no fish species found, 1 = non-predatory fish present, 2 = predatory fish present
Landscape variables	
Elevation	m a.s.l. (GPS)
Forest cover	% cover in a 800 m radius around the pond
Distance to forest	m
Green corridor	represents the connectivity between the pond and forest and is represented by a pasture/grass, brooks and hedgerows. 1 = present, 0 = absent
Pasture/grass cover	% cover in a 800 m radius around the pond
Arable land cover	% cover in a 800 m radius around the pond
High volume traffic road	asphalted roads 1 = present, 0 = absent

that denotes landscape configuration (Guerry and Hunter, 2002). We calculated land use composition around the ponds using the GIS software Manifold 7x, based on the CORINE Land Cover map (European Environment Agency 2006) occasionally supplemented with our visual estimations.

Data analysis

The predictor variables are continuous and discrete binary variables. Continuous variables were pond area, percentage of emergent aquatic vegetation cover, percentage of sallow water, distance of pond from the forest, the percentage of forest cover, percentage of pastures and grassland cover, percentage of arable land cover and elevation. Raw data were summarized by descriptive statistics. Due to the different scaling of the data the Coefficient of Variation (CV) was used as a dimensionless measure of variability, allowing an objective comparison among different variables.

The relatively low sample size (43 ponds), related to a large number of predictor variables does not allow reliable statistical inference due to the high dimensionality. Thus we opted for a multivariate approach. As the assumption of multivariate linearity of data (sensu Cox and Wermuth, 1994) is not met and the data set contains categorical as well as continuous variables, the nonlinear iterative NIPALS-PCA (Nonlinear Iterative Partial Least Squares-Principal Component Analysis) algorithm was used (Vandeginste et al., 1988). Raw data have substantially different ranges; consequently observations were transformed by mean centering and unit variance. The number of components extracted was determined from the eigenvalues (only those higher than 1 were selected). The possible existence of outliers that might bias the model was assessed by calculation of Hotelling's T^2 and Square of the predictions error (Q_i). How well a variable is represented by the PCA model is measured by the modelling power that runs from zero (irrelevant) to one (relevant). The extracted principal components then were related to the number of individuals recorded (adult toad counts). Due to the high differences in data structures a nonlinear relationship is likely, so the non-parametric Spearman correlation was used for this purpose. We also used one-way analysis of variance (ANOVA) to test for differences between ponds with or without toad populations in their factor scores. All statistical analyses were performed with the software Matlab 7.01.

Results

Common toads were present in 81.4% of the surveyed sites (fig. 1). Out of the 43 ponds sampled, 18.6% lacked fish, 30.2% had only non-predatory fish, while 51.1% contained both predatory and non-predatory fishes. 74.4% of the ponds are connected to forest through a green corridor, the mean cover of grass/pasture cover around the ponds is 32% and 37.2% of the ponds studied are close to roads with heavy traffic. Table 2 summarizes descriptive statistics of some aquatic and terrestrial environmental variables.

The average number of toads counted per site was 297 (95% CI: 116.2-431.1, upper and lower quartile: 100-300). Since the distribution of counts is skewed ($\gamma = 3.37$) and leptokurtic ($\beta = 13.6$), the 5% trimmed mean (196 individuals) probably offers a better estimation of the expected count values.

PCA analysis resulted in six new significant variables (PCA axes 1-6) that together explain 83.7% of the initial variance in count data (table 3). The last three principal components explain less than 10% of the initial variation and the sixth component has an eigenvalue smaller than one (table 3). We therefore consider only the first three axes. All variables are well represented by the principal components, the presence/absence of non predatory/predatory fish, green corridors and roads show the highest modelling power whereas the percentage of shallow water of

Table 2. Descriptive statistics for eight pond and landscape related variables (CV = Coefficient of Variation).

Variable	Mean	SD	CV
Area	99.212	319.266	3.22
Shallow water	34.49	27.7	0.80
Emergent aquatic vegetation cover	32.23	24.4	0.76
Elevation	395.37	72.1	0.18
Grass/pasture cover	32	20	0.63
Forest cover	34	22	0.65
Distance to forest	298.60	339.1	1.14
Arable land cover	15.8	20.2	1.27

Table 3. Characteristics of the first six principal components extracted from the 11 pond and landscape related variables used in this study ($R^2X =$ variance explained, $Q^2 =$ predictive variance).

Principal component	R^2X	R^2X (cum.)	Q^2	Q^2 (cum.)	Eigenvalues
PC1	32.59	32.59	28.27	28.27	4.89
PC2	17.02	49.60	45.25	60.72	2.55
PC3	11.53	61.14	56.22	82.80	1.73
PC4	8.48	69.62	64.25	93.85	1.27
PC5	7.54	77.16	73.72	98.38	1.13
PC6	6.53	83.69	81.82	99.71	0.98

Table 4. Factor loadings for the first three principal components on 11 pond and landscape related variables and the modelling power of each variable in the PCA analysis.

Variable	PC1	PC2	PC3	Power
Area	−0.081	−0.488	0.083	0.769
Shallow water	−0.031	0.692	−0.369	0.683
Emergent aquatic vegetation cover	0.426	0.368	−0.349	0.535
Non-predatory fish	0.103	0.439	0.762	0.969
Predatory fish	−0.192	−0.847	−0.211	0.923
Fish absent	0.125	0.570	−0.628	0.852
Elevation	0.417	−0.472	−0.032	0.817
Forest cover	0.555	0.229	0.342	0.880
Grass/pasture	0.152	−0.327	−0.361	0.856
Green corridor present	0.928	−0.174	0.026	0.900
Distance to forest	−0.880	−0.042	−0.079	0.816
Arable land	0.041	0.137	0.420	0.876
High volume traffic road	−0.904	0.030	0.085	0.889

the pond and emergent aquatic vegetation cover show the smallest but acceptable modelling power (table 4).

With respect to the relationships between factor loadings for each of the three principal components and toad presence and toad counts at each sampled site, the factor loadings for the first component (PC1) differ marginally between ponds with and without toads ($F_{[1,41]} = 3.29$, $P = 0.07$) and are significantly correlated with count data ($r_s = 0.35$, $P < 0.05$). On the other hand, loadings on the second ($r_s = -0.21$, $P > 0.05$) and third ($r_s = 0.27$, $P > 0.05$) PC axes were not significantly correlated with toad counts. Instead, PC2 highlighted the difference between ponds that were and were not used by toads for reproduction (PC2: $F_{[1,41]} = 9.7$, $P = 0.003$). Thus, sites characterized by high scores on PC1 (ponds located near forests and far from roads, with abundant aquatic vegetation and green corridors) are associated with both toad presence and larger counts, whereas sites with high scores on PC2 (ponds of relatively small size, shallow water and abundant aquatic vegetation where predatory fish are absent) are correlated with toad presence but not with their numbers.

Discussion

Understanding the ecological requirements and spatial habitat use by amphibians is crucial for the efficient conservation of this group (Semlitsch, 2001) and constitutes a major challenge to conservationists and natural resource managers. Since there may be large differences between different amphibian species regarding their habitat requirements and sensitivity to habitat loss and fragmentation (deMaynadier and Hunter, 1997; Kolozsvary and Swihart, 1999; Guerry and Hunter, 2002), species specific information is required to assess conservation status (Martinez-Solano et al., 2003; Cushman, 2006). Moreover, since *B. bufo* is a widespread species in Europe (Gasc et al., 1997), that uses several habitats, it is likely that population specific difference in factors affecting habitat use is present in distinct populations. This possibility also highlights the importance of studies carried out at different populations of this species.

The importance of aquatic related variables

Our study found no significant relationship between aquatic related variables and the Common Toad counts. However these variables explained pond occupancy by the common toad, suggesting they may be important in terms of habitat selection by this species. In a spatial model Halley et al. (1996) showed that both carrying capacity (as measured by pond size) and proximity to a source pond were important to maintain Common Toad populations. In our model, some pond characteristics like the presence of aquatic vegetation, presence of shallow water and absence of predatory fish are important determinants of pond occupancy by toads. Emergent macrophytes growing in the shallow, productive part of the ponds provide support for eggs (Hartel, 2004), shelter for adults and larvae, and a food-rich environment for larvae.

The Common Toad populations in this area appear to be unaffected by the presence of fish, although PC2 indicates that ponds used by toads for reproduction tend to contain both predatory and non-predatory fish. Predatory fish may have a positive effect on amphibians due to its differential predation on the different competitors and predators of some amphibian species, like dragonfly larvae (Smith et al., 1999; Maezono and Miyashita, 2003). The Common Toad larvae are able to cope with fish predation by secreting noxious substances (that makes them unpalatable), alarming substances (to alert conspecific individuals) and schooling behaviour (Manteifel and Reshetnikov, 2002). Similar results on this species were found by Martinez-Solano et al. (2003) and Orizaola and Brana (2006).

The importance of the landscape related variables

Many pond breeding amphibians, like the Common Toad, move across the landscape to reach the most important habitat resources, such as breeding, summering and wintering sites. Amphibians have low vagilities compared with other vertebrates, are in close contact with the ground, and have permeable skins that require certain microhabitat conditions (low temperature and moisture) for an efficient respiration and other physiological functions (Feder and Burggren, 1992). Due to these constraints, amphibians are sensitive to the changes in local conditions (i.e. microclimate) where they live, and to landscape fragmentation. Our results show that landscape elements related both to landscape composition and configuration are important in determining common toad population sizes. The Common Toad counts in our study area are positively associated with the presence of the green corridors between ponds and forests, the proximity of the forests to the breeding ponds and the amount of forested area around the breeding ponds. In the majority of cases the spatial arrangement of the habitat patches (pond connected to the forest by a green corridor and the proximity of pond to the forest, see table 2) in the landscape might allow toads to migrate and disperse safely between the critical habitat elements.

The multiple year surveys on some ponds in this area showed that no toad population extinctions occurred (Hartel, unpublished). The large, stable size of the toad populations and the large number of metamorphosing and dispersing juveniles (Hartel 2004; Hartel, unpublished), may have an important role in maintaining the Common Toad populations in a regional scale. Forest cover is high in the study area (33%), this habitat type being a major component of the landscape structure (see fig. 1). The positive effect of the proximity of breeding ponds to the forests on amphibians has been shown by many previous studies (i.e. deMaynadier and Hunter, 1995; Latham et al., 1996; Hecnar and M'Closkey 1998; Scribner et al., 2001). Moreover, amphibians could be present in high abundance in forests. Forests positively affect many amphibian populations by ensuring that conditions for feeding, moisture, shelter and hibernation for all terrestrial life stages.

The Common Toad has great dispersal ability in the juvenile stage (Reading et al., 1991) and the adults also show periodic migrations to reach the most important habitats, often separated in space (Sinsch, 1988). The characteristics of the matrix where these movements occur could either enhance or hinder dispersal and migration success. Species that have greater dispersal ability, such as the Common Toad, are exposed to dispersal mortality caused by changes in the matrix permeability within the landscape. The roads with high traffic density negatively affect toads. Roads modify the permeability of the habitat matrix, hampering the dispersal and migration of juveniles and adults (Ray et al., 2002). Massive road mortalities have been reported in several European amphibian species, including the Common Toad (Lodé, 2000), Common Spadefoot Toad (*Pelobates fuscus*) (Hels and Buchwald, 2001) and Moor Frog (*Rana arvalis*) (Vos and Chardon, 1998).

We did not find any significant effect of the percentage of arable lands surrounding the ponds on the Common Toad populations. In our study area only four ponds are completely surrounded by chemically treated cultivated lands, whereas in the majority of cases the land use is traditional, with natural fertilisers being used (personal observations).

Even if the present study shows no significant effect of the pond related variables on the Common Toads, many other amphibian species are significantly associated with the pond/aquatic habitat related variables. A recent study conducted in this area showed a positive association between six amphibian species and the emergent macrophyte cover, and a negative relationship between the pond use of four species and the introduced predatory fish (Hartel et al., 2007).

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