Travaux du Muséum National d'Histoire Naturelle «Grigore Antipa»	Vol. LIII	pp. 469–478	© Décembre 2010

DOI: 10.2478/v10191-010-0032-5

FACTORS INFLUENCING THE BREEDING HABITAT USE BY AMPHIBIANS IN THE ALPINE AREA OF THE RETEZAT NATIONAL PARK (ROMANIA)

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Abstract. We analyzed the distribution of amphibians according to the characteristics of breeding ponds in the alpine areas of the Retezat National Park. We inventoried 50 aquatic habitats between 1920 and 2260 m a.s.l. Three amphibian species are present at high altitudes: *Rana temporaria*, *Bufo bufo* and *Mesotriton alpestris.* They were found in 70% of the aquatic habitats, but only 48% were used as spawning sites. Human impacts and predators were the most influential variables explaining the occurrence of *R. temporaria*, whereas location and pond adjacent terrain were important for *M. alpestris*. Moderate grazing had a positive effect by maintaining low vegetation or providing nutrients input in the oligotrophic aquatic habitats.

Résumé. Nous avons analysé l'accessibilité des habitats et leur utilisation par les espèces d'amphibiens en deux zones situées à haute altitude dans le Parc National Retezat, qui occupent une superficie d'environ 2400 ha. Dans la zone étudiée (altitude 1920-2260 m) on a inventorié 50 habitats aquatiques. Les amphibiens (*Rana temporaria, Bufo bufo* et *Mesotriton alpestris*) sont présents dans 70% des habitats aquatiques inventoriés mais seulement 48% de ceux-ci sont utilisés pour la reproduction. Les variables qui influencent le plus la présence des espèces sont l'impact humain, la présence des prédateurs et les caractéristiques des habitats aquatiques pour *R. temporaria* et l'altitude et le terrain environnant dans le cas de *M. alpestris.* L'impact humain modéré peut avoir des effets bénéfiques par l'entrée de nutrients dans les lacs oligotrophes et en facilitant la dispersion des individus.

Key words: Amphibia, alpine area, habitat use, Retezat National Park, Romania.

INTRODUCTION

The alpine regions are some of the last undisturbed environments in Europe, but are still threatened by climate change and atmospheric deposition (Wathne & Hansen, 1997). Temperature increases lead to changes in alpine ponds hydroperiod and promote modification in the freshwater biota (Thuillier et al., 2005; Cannone et al., 2008). Because of their sensitivity, the freshwater communities are excellent systems for the study of the negative effects of climate change. Amphibians seem to be especially sensitive because of their biphasic life cycle. Amphibians have the highest proportion of species threatened with extinction among vertebrates, with more than 40% of species in decline (Stuart et al., 2004). They actively select habitats for breeding according to different aquatic and surrounding terrestrial landscape characteristics. Changes in the hydroperiod length could transform shallow permanent alpine lakes in temporary aquatic habitats, affecting the quality and proportion of available aquatic habitats. Some studies have already shown the effects of climate change on breeding phenology, reporting earlier breeding activities in amphibians (Terhivuo, 1988; Beebee & Griffiths, 2005).

Considering the rapid environmental changes that alpine landscapes are facing, it is important to understand the relationships between the aquatic habitats characteristics and their use by amphibians. These studies are important in developing conservation policies and for monitoring. In addition, high altitude landscapes are also impacted by changes in the grazing intensity (either intensification or decrease) and tourism. The impact of grazing on amphibians is still controversial, with some studies documenting its negative impact on amphibians (Schmutzer et al., 2008), others reporting a differential species-specific response (Burton et al., 2009), or even beneficial by removing vegetation in excess (Pyke & Marty, 2005).

High altitude areas in the Retezat National Park (RNP) have a complex structure, being modelled by past glaciation events and harbour a variety of permanent and temporary aquatic habitats. There is a low human impact, nevertheless grazing and trekking proved to have negative effects on vegetation (Mountford, 2006). The study area is situated at high altitudes and comprises two nearby zones with different levels of protection; one is the Scientific Reserve Gemenele – Negru, a strictly protected area, and the other is Zănoaga – Bucura, an area where controlled tourism and limited grazing are allowed. The aim of our research was to investigate the use and selection of aquatic habitats by amphibians in the alpine area based on environmental characteristics.

MATERIAL AND METHODS

Study area

The Retezat National Park (RNP) is the oldest protected area in Romania and one of the least human-affected protected areas in Central Europe (Fig. 1). It covers a surface of 38,138 ha and an altitude range between 794 and 2,509 m a.s.l.. The mean annual temperature varies between -2°C in the alpine area and 6°C in the valleys. The mean annual precipitation varies between 900 and 1,300 mm/year (Fărcaș & Sorocovschi, 1992).

The Scientific Reserve Gemenele is a strictly protected area with a surface of 1,630 ha. The Zănoaga-Bucura area is delimited by the Bucurelu, Porții, Zănoaga and Răsucit glacial lakes. The past management of the area had alternating periods when grazing was allowed, regulated or prohibited. The two areas cover together a surface of 2,400 ha and have an altitudinal range between 1,920 and 2,260 m a.s.l. (Fig. 1). All the investigated aquatic habitats are located above the tree line.

The aquatic habitats and amphibians were inventoried during the period 2000–2006. The geographic coordinates and altitude of each aquatic habitat were recorded with a handheld Garmin GPS device. The water temperature, pH and conductivity values were measured with a portable Oakton Waterproof pH/mV/C Meter. Each site was characterized by a number of aquatic and terrestrial (landscape) variables (Tab. 1).

Three amphibian species occur within the study area: *Rana temporaria*, *Bufo bufo* and *Mesotriton alpestris*. A single female of *Bombina variegata* was found in the area, but no breeding population exists above the tree line. Amphibians were inventoried based on visual transects on land and dip-netting in water. All life stages (eggs, larvae, juveniles and adults) were considered.

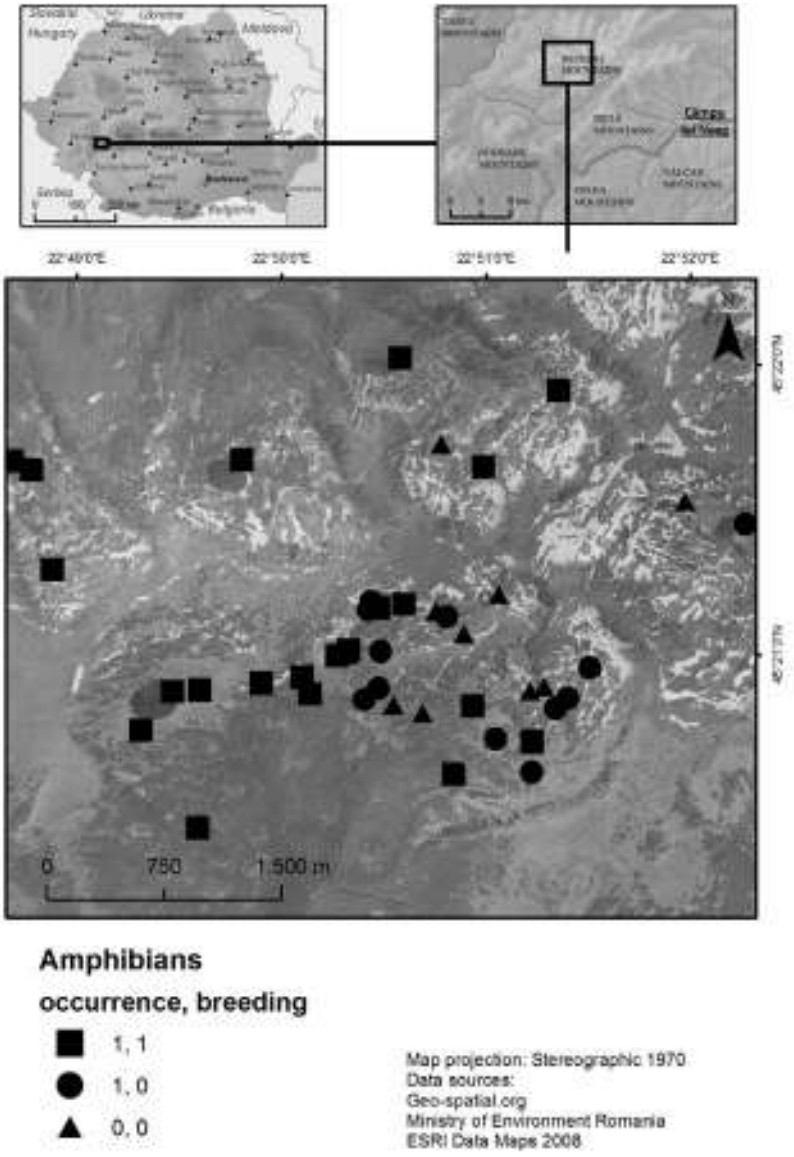

Fig. 1 - The location of sample sites in the RNP (Retezat National Park).

Data analysis

All continuous variables were *z*-transformed (standardized to an average of zero and a standard deviation of one) in order to allow comparability of predictors' effect (Ćirović et al., 2008). Correlated variables were subject to a Principal Component Analysis (PCA). Uncorrelated variables and first PCA axes were used to build 12 a priori models. The models were built by considering that different local

Table 1

The variables scored for each inventoried aquatic habitat.

Category of variables	Variable	Covariate
Localization (Loc)	Latitude (Lat) Longitude (Long) Altitude (Alt) (m)	
Aquatic habitat	Pond permanency (Pp) Pond depth (D) (cm) Pond surface (S) (m^2) pH Conductivity (C) (μs/cm) Water temperature (Wt) (^{0}C) Fish (PF) Invertebrate predators (Prd)	Permanent pond; temporary pond presence; absence presence; absence
Landscape	Adjacent terrain (100 m radius) Human impact (Grazing)	Pasture (Mdw); Scree (Scr); Dwarf pine (Dp) presence; absence

and landscape processes induced different associations between the species distribution and habitat features (Van Buskirk, 2005). The details about the model selection approach and the ecological background of similar models were presented in Johnson & Omland (2004) and Van Buskirk (2005). We used generalized linear models (GLMs) to relate the explanatory variables (pond and landscape characteristics) to the binary coded response variable (species occurrence). A binomial error distribution (logit regression) was assumed to relate the binary response variable and continuous explanatory variables. Akaike information criterion (AIC) was used to select the best model and rank the remaining models and "model averaging" to calculate the parameter estimates (Tab. 3). For each model, the AIC value was calculated using correction for small samples sizes (AICc) (Burnham & Anderson, 2002). Model averaging was performed with AICcmodavg package (Mazerolle, 2009) and R software (R Development Core Team, 2009).

RESULTS

We inventoried 50 aquatic habitats of which 32 were permanent glacial lakes (Fig. 1). The aquatic habitats were surrounded by grassland, scree or alpine shrubs. All aquatic habitats were of natural origin, oligotrophic, with a depth ranging between 0.1 and 29 m (Tab. 2). Amphibians were present in 70% of the aquatic habitats, but only 48% were used for reproduction. The most common species were *Rana temporaria* with 68% pond occupancy and the largest altitudinal range, followed by *Mesotriton alpestris* with 28%, and *Bufo bufo* with only 10% pond occupancy (Fig. 2). Because of its low frequency of occurrence, *B. bufo* was not included in the following analyses.

Table 3 summarizes the model selection results and table 4 presents the parameter estimates of the used covariates. The best model for predicting *R. temporaria* and *M. alpestris* occurrences was the human impact and location model, respectively. Akaike parameter weights indicated that the human impacts and

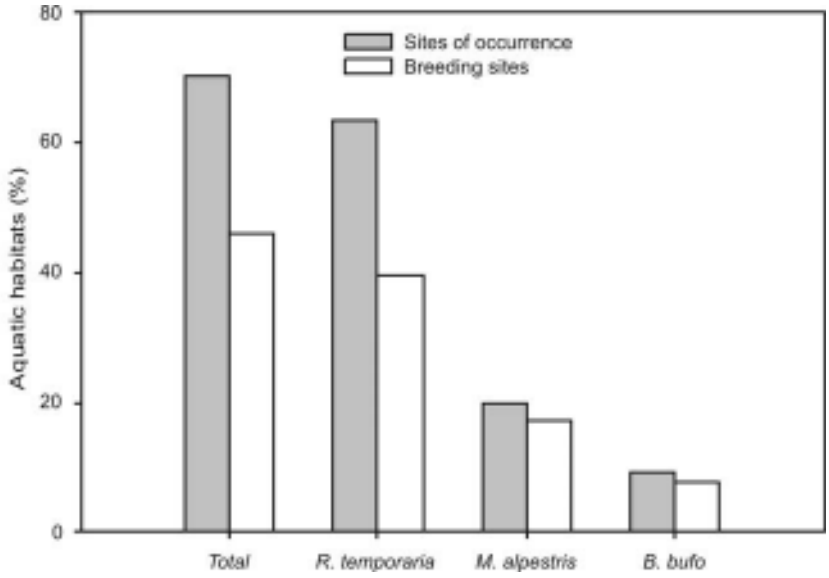

Fig. 2 - Occurrence and breeding of amphibians in the investigated aquatic habitats.

Table 2

The characteristics of the aquatic habitats in the alpine area (RNP).

	Average	Standard Deviation	Range (min-max)
Altitude (m)	2071.7	70.5	1920 - 2260
Water surface (m^2)	4459.3	11051.3	12.6 - 60000
Depth (cm)	207.7	532.9	10 - 2900
Water temperature (^oC)	12.5	3.3	5.3 - 20.1
pH			4.6 - 8.5
Conductivity (µS/cm)	12.7	4.1	5.6 - 25.5

predators were the most influential variables of *R. temporaria* occurrence, whereas the location and pond adjacent terrain were important for *M. alpestris*.

DISCUSSIONS

Amphibians use aquatic habitats differently in relation to landscape and aquatic habitat characteristics. Localization, hydroperiod, landscape characteristics, particularly the availability of terrestrial refugia were found to be important in determining the suitability of the breeding sites for *M. alpestris* and *R. temporaria* (Marnell, 1998; Bosch & Martinez-Solano, 2003; Van Buskirk, 2005; Hartel et al., 2010). Although the study area had a high density of aquatic habitats, the level of pond occupancy was low, probably due to the isolation of some water bodies. Even if each species has specific habitat requirements, the variables used in the study were of low discriminative power. Since the studied amphibian species have a

Table 3

The model selection results for predicting the occurrence of *R. temporaria* and *M. alpestris* in ponds from the Retezat National Park. Statistics include the number of estimated parameters (K), the second order Akaike Information Criterion (AICc), AIC difference (Δi), and Akaike weights (*wi*). The values in bold indicate the best model and the models with substantial weights (delta (Q)AIC(c) ≤ 2). Where Loc - first PCA axis on Alt, Lat and Long; Wch - First PCA axis on pH and C; PR - first PCA axis on PF and Prd; HP - First axis PCA on S, D, Pp, Tw.

Models	Covariates	*R. temporaria*				*M. alpestris*		
		K	AICc	Δi	*wi*	AICc	Δi	*Wi*
1	Loc, HP, Wch, PR, Mdw, Dp, Scr, HI	9	78.111	13.459	0.000	69.102	8.818	0.003
2	Loc	2	66.774	2.122	0.080	**60.284**	**0.000**	**0.267**
3	HP	2	**66.110**	**1.458**	**0.111**	63.453	3.169	0.055
4	Wch	2	66.899	2.247	0.075	63.445	3.161	0.055
5	PR	2	**65.838**	**1.186**	**0.127**	62.737	2.453	0.078
6	Wch, HP, PR	4	70.038	5.386	0.016	65.765	5.481	0.017
7	Mdw, Dp, Scr	4	69.720	5.068	0.018	**60.566**	**0.282**	**0.232**
8	Wch, HP	3	68.099	3.447	0.041	65.661	5.377	0.018
9	Wch, PR	3	67.911	3.259	0.045	64.696	4.412	0.029
10	HI	2	**64.652**	**0.000**	**0.230**	63.401	3.117	0.056
11	HI, Mdw, Dp	4	67.146	2.494	0.066	**61.262**	**0.978**	**0.164**
12	HI, PR	3	**65.033**	**0.380**	**0.190**	64.949	4.665	0.026

Table 4

The coefficients of covariates predicting the occurrence of *R. temporaria* and *M. alpestris* in ponds from the Retezat National Park that were obtained by model averaging.

Covariates	*R. temporaria*	*M. alpestris*
HI	1.074	0.149
Mdw	-0.091	0.428
Dp	0.047	-0.929
Scr	0.103	-0.683
Loc	-0.123	0.538
HP	0.332	-0.213
Wch	-0.119	-0.123
PR	0.416	0.332

metapopulation structure, their distribution may change in time because of metapopulation functioning (Skelly et al., 1999).

Mesotriton alpestris

The alpine newt can tolerate cold temperatures being considered as a temperate-climate euryeceous species (Griffiths, 1996). However, the pond localization was an important factor in this study, influencing the occurrence of species, its altitudinal range in this area being lower than *R. temporaria*'s. In our study the landscape variables were more important for this species than the aquatic

habitats characteristics. The specific preferences of species are masked in harsh environments, the animals being constrained to use available resources (Bosch & Martinez-Solano, 2003). Scree and dwarf pine may play an important role in newt distribution by increasing the difficulty of migration, while pastures may facilitate dispersion and could represent an important habitat for terrestrial stage feeding.

The alpine newt is negatively affected by fish (Joly et al., 2001; Orizaola & Brana, 2006; Denoël & Ficetola, 2008); nevertheless in our study this factor does not have a significant importance. Generally, predator fish was introduced in the study area in lakes deeper than 3 to 4 m. We found this species in shallow aquatic habitats, with only three lakes deeper than 1 m.

Rana temporaria

This species is the only one reaching high elevation lakes in RNP and avoids aquatic habitats with fish. Previous field observations reported negative impact of fish on *R. temporaria* populations (Meyer et al., 1998). Experimental studies showed that the larvae displayed behavioural responses in the presence of fish chemical cues (Nyström & Åbjörnsson, 2000), but could not coexist with predatory fish (Meyer et al., 1998; Nyström & Åbjörnsson, 2000). In our study, the aquatic habitat characteristics were more important for this species than landscape variables, highlighting the importance of aquatic habitats for reproduction and overwintering habitat. The adults use aquatic habitat as overwinter habitat but water should be deep enough not to be affected by the frost or by lack of oxygen; otherwise mass-mortality could occur (Cogălniceanu & Hartel, 2005).

The human impact model showed a positive correlation between grazing and the occurrence of both species. Grazing is usually considered to have a negative impact on amphibians at low altitudes (Knutson et al., 2004; Schmutzer et al., 2008; Burton et al., 2009). Grazing in the alpine areas could have a positive effect by maintaining low vegetation or facilitating dispersion and by providing a nutrient input to the oligotrophic aquatic habitats. Nevertheless this is not a major variable shaping the amphibian communities in the studied areas because the same species are present also in the strictly protected area, where grazing has been prohibited since 1960.

Based on trends in past daily temperatures from high altitude meteorological stations, Micu & Micu (2008) estimated that winters would become warmer and drier in the Carpathians. In the light of predicted climate change, it is expected that alpine species will adjust behaviourally and physiologically to the new conditions of their habitats (Carey, 2005). Understanding the aquatic habitat features and the local breeding habitat preferences of amphibians is a starting point for comparative studies that will allow developing predictive models on the effects of climate change on amphibians that inhabit the alpine area. In a harsh environment like the studied alpine area, species distribution is mostly limited by environmental factors and there is little habitat choice. The low human impact in the area is not detrimental to the persistence of amphibian populations. The predicted climate changes will nevertheless impose shifts in habitat use and will require constant monitoring for adaptive management. Actually, low grazing and prohibited stocking with predatory fish represent the main management measures for amphibian protection in the area.

ACKNOWLEDGEMENTS

Funding was provided by the CNCSIS Grant No. 1114/2004. The Centre of Study and Research for Agrosylvicultural Biodiversity “David Davidescu”, the Administration of Retezat National Park and the Commission for Nature Conservation of the Romanian Academy provided

access permits and logistic support in the field. We are deeply indebted to Dr. Tibor Hartel for carefully revising the manuscript and for improving the paper.

FACTORI CE INFLUENȚEAZĂ UTILIZAREA HABITATELOR ACVATICE DE CĂTRE COMUNITĂȚILE DE AMFIBIENI DIN ZONA ALPINĂ A PARCULUI NAȚIONAL RETEZAT (ROMÂNIA)

REZUMAT

Modul în care speciile utilizează habitatele este important pentru conservarea biodiversității. Populațiile de amfibieni ce ocupă habitate în zone situate la altitudini ridicate sunt mai vulnerabile la schimbările climatice decât cele de la altitudini scăzute. Am analizat accesibilitatea habitatelor acvatice şi utilizarea acestora de către speciile de amfibieni în două zone situate la altitudini ridicate în Parcul Național Retezat ce ocupă o suprafață de aproximativ 2400 ha. În zona studiată (altitudine 1920-2260 m) au fost inventariate 50 de habitate acvatice. Cele trei specii de amfibieni (*Rana temporaria*, *Bufo bufo* şi *Mesotriton alpestris*) sunt prezente în 70% din habitatele acvatice inventariate, dar numai 48% dintre acestea sunt utilizate pentru reproducere. Variabilele care influențează cel mai mult prezența speciilor sunt impactul uman, prezența prădătorilor şi caracteristicile habitatelor acvatice pentru *R. temporaria* şi altitudinea şi zona terestră limitrofă habitatelor acvatice în cazul lui *M. alpestris*. Impactul uman moderat reprezentat de păşunatul alpin poate avea efecte benefice prin intrările de nutrienți în lacurile oligotrofe şi prin facilitarea dispersiei indivizilor.

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Received: April 2, 2010
Accepted: October 11, 2010

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