# BREEDING SITE TRAITS OF EUROPEAN NEWTS (TRITURUS MACEDONICUS, LISSOTRITON VULGARIS, AND MESOTRITON ALPESTRIS: SALAMANDRIDAE) IN THE MONTENEGRIN KARST REGION

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Abstract — We recorded the occurrence of three European newt species - the smooth newt (*Lissotriton vulgaris*), the eastern alpine crested newt (*Triturus macedonicus*), and the alpine newt (*Mesotriton alpestris*) - in the Montenegrin karst, as well as their breeding site characteristics. In terms of long-lasting breeding site numbers and occupation rate, the most common species is the smooth newt, followed by the alpine newt and the crested newt. The examined water bodies without newts showed no significant differences of aquatic habitat characteristics compared to water bodies with newts. The factors that explained most of the observed variation in newt breeding site traits were the habitat category and habitat origin. The alpine newt primarily inhabits natural lakes, while the crested newt inhabits artificial breeding sites such as lithotelma and ubao. The smooth newt is less choosy and occurs in different types of natural and artificial habitats. The aquatic requirements of Montenegrin newt species do not differ substantially in many respects from requirements of the core species range populations.

Key words: Smooth newt, alpine newt, crested newt, holokarst, allotopy, syntopy, conservation, Montenegro

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### INTRODUCTION

European newts (*Lissotriton* sp., *Triturus spp.*, and *Mesotriton sp.*) use a variety of water bodies that differ in an array of abiotic factors (e.g., pond water depth, water chemistry, pond isolation), as well as biotic factors (e.g., presence of competitors and predators and effects of their interactions, potential mate presence, etc; Jakob et al., 2003). The quality and quantity of these characteristics partially determine the presence of newt populations to the extent that some features can be isolated as being of particular diagnostic importance and used as a basis for determining the likelihood of newts' occurrence (Oldham et al., 2000).

Freshwater lentic habitats range from ephemeral puddles to large permanent lakes, with concomitant gradients in many features that covary with size and permanence of the water body (Van

Buskirk, 2003). Temporary ponds provide a rich (in terms of food resources) but transient environment for amphibians (Griffiths, 1997) and are a major breeding habitat for amphibians in the Mediterranean region (Diaz-Paniagua, 1990). However, amphibian breeding sites with a mostly stable hydroperiod, which are by far the predominant type of water bodies in the Montenegrin karst region, have escaped investigations so far.

Available data on newts' habitat selection primarily refer to populations from Western, Northern, and Central Europe (Cooke and Frazer, 1976; Swan and Oldham, 1994; Marnell, 1998; Sztatecsny et al., 2004; Skei et al., 2006). Much less is known about populations from Eastern Europe (Babik and Rafinski, 2001) and the Western Mediterranean (Pavignano et al., 1990; Ildos and Ancona, 1994; Denoël and Lehmann, 2006), and only anecdotal data are available on

ones from Southeaste Europe, including the Balkan Peninsula (Bousbouras and Ioannidis, 1997; Denoël, 2004, Kati et al., 2007). Objective methods of measuring and monitoring newt populations have never been applied in the Dinaric karst regions. The current information on newt breeding sites in this area is scarce and limited to narrow territories (Džukić, 1981; Kalezić and Džukić, 1985; Džukić and Kalezić, 1988; Bruno, 1988). What is more, no studies so far have examined simultaneously the factors that affect the distribution of newts in the Dinaric karst regions.

Our primary objective was to assess patterns of variation in European newt breeding site characteristics in the Montenegrin karst area and search for possible association(s) between aquatic habitat traits and the presence of particular species. We studied all newt species living in this region: the smooth newt (Lissotriton vulgaris), the eastern alpine crested newt (Triturus macedonicus, hereinafter referred to simply as the crested newt), and the alpine newt (Mesotriton alpestris). The Montenegrin karst area is relevant in several ways for this study. Firstly, as noted below, this region is inhabited by endemic or subendemic taxa and/or phylogenetic clades of each of the newt groups, whose breeding site characteristics have never been assessed systematically. Secondly, the examined region is on the range margins of all three newt groups (Griffiths, 1996), thus harboring geographically (and most likely ecologically) peripheral populations (Lesica and Allendorf, 1995; Araújo and Williams, 2001).

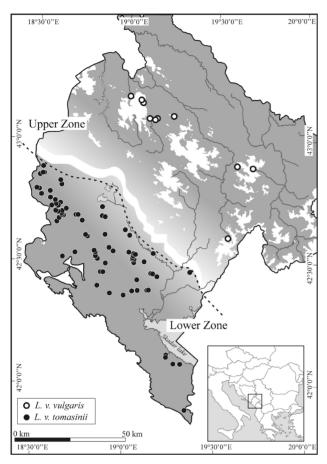
In a previous study of ours, we determined European newt distribution patterns in the Montenegrin karst (Ćirović et al., submitted). Briefly, the smooth newt occurred throughout the Montenegrin karst region, except in the most elevated parts. The alpine newt was restricted mostly to the Upper Zone, while the crested newt was represented the least, occupying only a part of the Lower Zone (Figs. 1 and 2). In addition, using land-scape traits obtained by combining remote sensing and geographic information systems, we found that geographic position (latitude and longitude) and (especially) elevation have a great impact on newt occurrence.

The aims of this paper are twofold. To begin with, we present for the first time an account of breeding site characteristics of Montenegrin karst area newts. Secondly, based of this analysis, we sought to answer (1) whether some of these breeding site characteristics shape the observed distribution pattern of European newts in the Montenegrin karst region, and (2) do the newt species differ much in their aquatic requirements when they inhabit fringe areas of the taxon's distribution?

#### MATERIALS AND METHODS

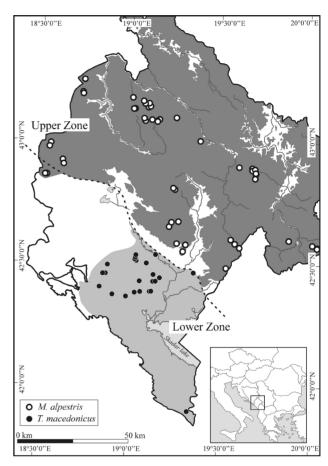
#### Study area

The segment of the Dinaric Alps which forms the terrain of Montenegro is predominantly built of limestone and dolomite sediments that have been exposed to various forms of karstification (Cvijić, 1989). The karst zoning of Montenegro is very complex (Radulović and Radulović, 1997). However, for the sake of simplicity with regard to our investigation, the karst region can be divided into two zones separated mainly by the spacious Zeta depression, where flysch deposits are found. The landscape of the northern Upper Zone is an exclusively mountainous region, covered with forests and grasslands, with little bare karst. Short spring-fed watercourses are relatively common, but lakes of various sizes, mostly of glacial origin, as well as different kinds of ponds, are the main amphibian (for newts almost the only) breeding sites in this zone. On the whole, these water bodies are not densely distributed. The landscape of the southern Lower Zone is a less elevated region with bare karst (called holokarst) over its largest part. Most of this is exposed karst with scanty plant growth in the form of crack dwellers. The Lower Zone is a flowing-water-free karst region. The limestone base of the zone, being highly porous, drains much of the rainfall, causing extreme aridity during the summer months. Relatively scarce surface water bodies, usually at the sites of sinkholes and potholes with impermeable bottoms, in which water comes almost exclusively from rainfall and rarely from boiling springs, are the only ones available for amphibian reproduction. These bodies have been used since a very distant past as cattle drinking water or even



**Fig. 1.** Range of distribution of *L. vulgaris* in Montenegro (shaded areas) and geographic position of *L. vulgaris vulgaris* and *L. vulgaris tomasinii* breeding sites used in this study. Dashed line delimits the Upper and Lower Zones.

for human consumption. As water was constantly in short supply, people have always taken great care of these ponds. By building dams or walls, as in the case of wells, they have enabled these ponds to last a long time. Thus, the holokarst is characterized by for the most part permanent ponds, the majority being artificial or partly artificial. These ponds vary in the hydroperiod not only spatially, but also temporally, and newt species have to cope with unpredictable annual fluctuations within the ponds. Rarely, some of them dry up due to severe prolonged droughts, thus preventing any newt metamorphosis. Overall, our study includes two general landscapes (Lower and Upper Zones) that differ in many respects, such as elevation, climate, water body characteristics,



**Fig. 2.** Ranges of distribution of *T. macedonicus* (light gray) and *M. alpestris* (dark gray) and geographic position of their breeding sites in Montenegro. Dashed line delimits the Upper and Lower Zones.

vegetation types near the water bodies, the extent of karstification, and, concomitantly, the extent of population connectivity.

## Studied newt taxa and distribution patterns

Four newt taxa inhabit the Montenegrin karst area (Figs. 1 and 2). Among smooth newts, individuals of the nominotypical subspecies (*L. vulgaris vulgaris*) are encountered in the northern parts of the Upper zone and extend up to 1700 m, while the endemic Southern Adriatic smooth newt (*L. vulgaris tomasinii*; Krizmanić et al., 1997) occurs throughout the Lower zone, as well as along the Zeta River depression. Noteworthily, the Montenegrin ranges of these taxa are separated

by a smooth newt-free zone. Of five crested newts (*Triturus cristatus* superspecies), the eastern alpine crested newt *Triturus macedonicus*, an endemic species of the central Balkans, lives in part of the Lower Zone and in the Zeta River depression. This newt has been recently elevated to species level by Arntzen et al. (2007). Populations of the alpine newt's phylogenetic distinct clade endemic to Montenegro (Sotiropoulos et al., 2007) are restricted to the northern mountainous part of the country. The taxonomic status of this clade has not yet been clarified.

Apparently, in terms of associate distribution, the Montenegrin karst region is inhabited by two newt taxa groups – the southern *L. vulgaris tomasinii* and *T. macedonicus*, and the northern *M. alpestris* and *L. vulgaris vulgaris*. Within both groups, threre is a partial sympatry with frequent syntopy. A sharp range boundary, which runs along the foothills of the Upper Zone, separates in a clear-cut manner their northern and southern ranges, respectively.

### Data obtained

Research was carried out over the period of 2002-2005 at historically fishless 142 breeding sites. Our survey covered an area of about 10080 km². The identified breeding sites were visited during at least two spawning and two breeding seasons. The visual encounter survey or VES (Crump and Scott, 1994) method, which is effective for most species that breed in lentic (non-flowing) waters, was the most frequently used technique throughout the study. Each aquatic site was surveyed for 45 minutes. Most newts were identified by sight, but dip nets were used in areas with vegetation or where water clarity was poor.

The following six aquatic habitat variables were scored at each sampled site: I. habitat origin (1. natural; 2. artificial), II. habitat category (1. ubao; 2. pond; 3. lake; 4. river overflowing; 5. kamenica (lithotelma); 6. pool; for terminology see Gavrilović, 1974; Cvijić, 1989), III. water permanency (1. permanent water with narrow water level oscillations; 2. permanent water with significant water level oscillations; 3. spring-time water (occurs during the spring

months only); 4. ephemeral water (occurs during rainfalls and quickly dries out), IV. water vegetation (1. macrophyte only; 2. algae only; 3. macrophyte and algae; 4. without vegetation), V. presence of fish (1. present; 2. not present), and VI. pH level (1.  $6 \le pH < 7$ ; 2.  $7 \le pH < 8$ ; 3.  $8 \le pH < 9$ ; 4.  $9 \le pH < 10$ ; 5.  $10 \le pH < 11$ ). Acidity was measured with a pocket pH meter having a measurement error of 0.01 pH. The presence of fish and water permanency were determined by visual surveys and from information gathered by questioning the local village population.

# Statistical analyses

Six groups of sites were formed regarding the occurrence of (1) *T. macedonicus*; (2) *L. vulgaris*; (3) *M. alpestris*, (4) *L. vulgaris* and *T. macedonicus*, (5) *M. alpestris* and *L. vulgaris*, and (6) without the occurrence of newts.

Prior to further analyses, correlations between the aquatic habitat variables were tested with the Spearman rank test to eliminate unwanted variable overflow. The frequency of occurrence (%) of aquatic habitat categories was calculated for sites with newts taken as a whole, and for each of the six groups of sites individually. In order to summarize the data into a minimal number of variables, a detrended correspondence analysis (DCA) was performed using PC-ORD v. 4.25 software. This technique was chosen over other ordination techniques so as to prevent, the data from being nonlinear (cf. principal components analysis), avoid the arch effect produced when plotting the major axes of variation (cf. correspondence analysis), and take advantage of DCA's ability to utilize binary variables (cf. multidimensional scaling) (Digby and Kempton, 1987). Prior to DCA, data set relativization for each variable maximum was performed. Spearman rank correlations were calculated to examine possible correlation between the aquatic habitat variables and the DC site scores. The DC site scores were subjected to the nonparametric Mann-Whitney test in order to assess the existence of group differences (analyses were performed with Statistica 5.5 software from StatSoft Inc.). Due to the small number of breeding sites with *T. macedonicus* (only three sites), the DCA was performed without them.

## **RESULTS**

## Newt occurrence patterns

Newts were present in 91.6% of the studied sites, proving them to be the most conspicuous batrachofaunal element of the karst region. As such, European newts are candidates for being the primary ecological group in further monitoring programs of the Montenegrin karst area. The most common species at the examined breeding sites was the smooth newt, with occupancy of 70.0%, followed by the alpine newt (36.9%) and the crested newt (17.7%).

At most spawning sites (75.4%), newt species did not share the same water body during reproduction, with the lowest percentage prone to allopatry in the crested newt (13.0 %), followed by the smooth newt (69.8%) and the alpine newt (75.0%). Newt species assemblages consisting of individuals of two species were found at 24.6% of all the examined sites with newts; syntopy of *T. macedonicus* and *L. vulgaris* occurred in 62.5% of cases and syntopy of *M. alpestris* and *L. vulgaris* in 37.5%, while *T. macedonicus* and *M. alpestris* were not found to coexist at the same breeding site.

**Table 1.** Frequency of occurrence (%) of aquatic habitat categories for Montenegrin newts. The numbering is explained in "Material and Methods". N – Sample size.

		T. macedonicus	L. vulgaris	M. alpestris	L. vulgaris + T. macedonicus	M. alpestris + L. vulgaris	with newts	without newts
Variable	Category	N=3	N=59	N=36	N=20	N=12	N=130	N=12
Habitat origin	1	33.3	23.7	77.8	25.0	83.3	44.6	41.7
	2	66.7	76.3	22.2	75.0	16.7	55.4	58.3
Habitat category	1	33.3	44.1	19.4	45.0	8.3	33.8	58.3
	2	33.3	25.4	5.6	15.0	33.3	19.2	8.3
	3	0.0	0.0	69.4	5.0	58.3	25.4	25.0
	4	0.0	0.0	0.0	5.0	0.0	0.8	0.0
	5	33.3	28.8	5.6	30.0	0.0	20.0	8.3
	6	0.0	1.7	0.0	0.0	0.0	0.8	0.0
Water permanency	1	100.0	59.3	77.8	50.0	66.7	64.6	66.7
	2	0.0	33.9	22.2	45.0	16.7	30.0	25.0
	3	0.0	5.1	0.0	5.0	16.7	4.6	8.3
	4	0.0	1.7	0.0	0.0	0.0	0.8	0.0
Water vegetation	1	33.3	6.8	2.8	10.0	8.3	6.9	0.0
	2	66.7	32.2	22.2	35.0	25.0	30.0	41.7
	3	0.0	55.9	72.2	55.0	66.7	60.0	33.3
	4	0.0	5.1	2.8	0.0	0.0	3.1	25.0
Presence of fish	1	0.0	8.5	44.4	5.0	50.0	21.5	25.0
	2	100.0	91.5	55.6	95.0	50.0	78.5	75.0
	1	0.0	0.0	5.6	0.0	0.0	1.5	0.0
рН	2	33.3	11.9	33.3	15.0	58.3	23.1	33.3
	3	0.0	61.0	47.2	65.0	33.3	53.8	66.7
	4	66.7	25.4	11.1	20.0	8.3	20.0	0.0
	5	0.0	1.7	2.8	0.0	0.0	1.5	0.0

**Table 2.** Spearman correlations (rs) with significance probabilities (P) among pertinent aquatic habitat variables and DCA ordination site scores on the first (DC1) and second (DC2) ordination axis.

Aquatic habitat variables	D	C1	DC2		
	r <sub>s</sub>	P	r <sub>s</sub>	P	
Habitat origin	-0.72	0.0000	0.13	0.1333	
Habitat category	0.90	0.0000	-0.05	0.5686	
Water permanency	0.26	0.0019	0.61	0.0000	
Water vegetation	0.21	0.0121	-0.72	0.0000	
Presence of fish	-0.45	0.0000	0.36	0.0000	
pH	-0.05	0.5875	0.38	0.0000	

A notable characteristic of newt distribution patterns was the existence of sharp boundaries, i.e., the western and northern margins of distribution of the crested newt and the southern margin of distribution of the alpine newt (see Fig. 2).

#### Habitat characteristics and newt occurrence

When altitude range patterns were compared, the smooth newt and the crested newt were found to have the greatest variation (from sea level up to 1700 m), while the alpine newt is confined to terrains above 870 m.

Since the Spearman rank test showed a lack of correlations between aquatic habitat characteristics, except for a moderate correlation between the habitat category and origin ( $r_s = -0.51$ , P < 0.001), all characteristics were included in further analyses.

The frequency of occurrence (%) of aquatic habitat categories showed that newts in the Montenegrin karst were found in both natural and artificial fishless habitats, usually in ubao, lakes, or lithotelma with permanent alkaline water (pH between 8 and 9) and narrow water level oscillations, inhabited by both macrophytes and algae (Table 1).

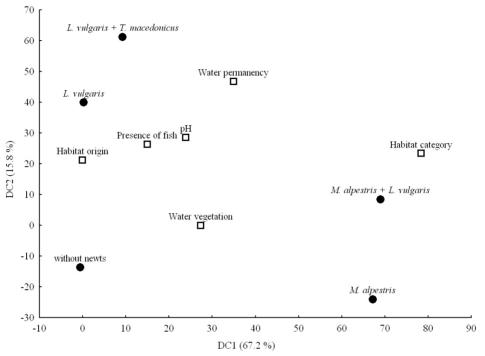
DCAs were performed in order to find potentially significant connections between breeding site characteristics and newt occurrence. The first and the second DC axis explained 67.2 % and 15.8 % of total variation, respectively. The highest scores on the first axis were for habitat category, followed by water permanency and type of water vegetation (Fig.

**Table 3.** Comparison of DC1 and DC2 site scores with Mann-Whitney U test.

\*\*\*P<0.001; \*\*P<0.01; \*P<0.05; ns - non significant.

		1.	2.	3.	4.	5.
1. L. vulgaris						
2 M -15 tui-	DC1	**				
2. M. alpestris	DC2	**				
2 I mileania/T magadaniana	DC1	ns	*			
3. L. vulgaris/T. macedonicus	DC2	ns	***			
A. M. alpastuis/I. mulaguis	DC1	*	ns	*		
4. M. alpestris/L. vulgaris	DC2	ns	ns	ns		
5. without newts	DC1	ns	ns	ns	ns	
5. Without flewts	DC2	ns	ns	*	ns	

3). The highest scores on the second axis were for water permanency, followed by pH, presence of fish, and habitat origin (Fig. 3). However, only habitat category and origin were significantly related with DC1, and water permanency and vegetation with DC2, which had a high impact on site segregation along the first and the second DC axes (Table 2). Two groups of localities were discernible along the first DC axis: a group of localities with M. alpestris (in allotopy and syntopy) on one side; and a group of localities with L. vulgaris, localities with L. vulgaris and M. macedonicus, and localities without newts on the other side (Fig. 3). Alpine newts were primarily found in lakes (natural origin), while the other two newt species were mostly found in ubao or lithotelma (artificial origin) (Table 1). Localities with smooth newts in allotopy and syntopy with crested newts were segregated along the second axis due to a high proportion of water bodies with significant water level oscillations (Table 1). Sites without newts were detached from other groups along DC2 due to a moderate proportion of sites without vegetation (Table 1). Analyses of site scores showed significant differences between groups along the first axis and, to a lesser extent, along the second axis (Table 3). The most notable finding of our study is that there are no breeding site attributes that clearly discriminate localities with newts and localities without newts, in view of both DC1 and DC2 scores (Table 3). An exception was a significant discrepancy between the group of localities without newts and the group of localities with L. vulgaris and M. macedonicus along the DC2 axis due to differences of water permanency (see Table 1).



**Fig. 3.** Plot of newt breeding sites and aquatic habitat scores on the first and second detrended correspondence axes (DC).

## **DISCUSSION**

The adaptability of European newts to different spawning sites would appear to vary from species to species. It is commonly held that crested newts have habitat requirements that are more demanding than those of most other newt species (Arntzen and Teunis, 1993). For these newts, water bodies with a long hydroperiod, high macrophyte density, and terrestrial habitat diversity have been indicated as especially important (Oldham et al., 2000; Babik and Rafinski, 2001). In ecological terms, the western alpine crested newt (T. carnifex) from Calabria (Italy) is regarded as a mesophilic or orophilic (mountain-dwelling) species, mostly found in ponds rich in hygrophilic vegetation and located in open spaces (Giacoma et al., 1988). Its phylogenetic closely related species in the Montenegrin karst does not appear to have different aquatic requirements, as far as our study shows.

The smooth newt is a species with a relatively wide ecological amplitude (Dolmen, 1988, and references therein). Associations between aquatic

vegetation and the presence of this species seem to be a species characteristic valid throughout most of the species range (Ildos and Ancona, 1994; Marnell, 1998; Babik and Rafinski, 2001). We have also shown that this species, being in allotropy and syntopy with two in many ecological aspects different species (alpine newt and crested newt), behaves as an opportunist in ecological demands regarding the characteristics of breeding sites.

The alpine newt is ecologically defined as a temperate-climate euryeceous species which is quite resistant to cold temperatures, but not to hot and dry conditions (Griffiths, 1996). However, we found this newt in spawning sites within a restricted area of the Upper Zone of the Montenegrin karst region, which has a karst terrain with Mediterranean climate (950 m a.s.l.). It is worth mentioning that the alpine newt is found in other areas of exposed karst in the Balkans, but at a much lower altitude (i.e., Mt. Velebit, Croatia, 80 m a.s.l., Kalezić et al., 1990).

As far as breeding sites are concerned, the aquat-

ic preferences of Montenegrin karst newts overall appear to fit the general picture of "preferred" newt breeding sites elsewhere (water bodies with a relatively stable hydroperiod, dense aquatic vegetation, and intermediate pH and ionic concentration, but without fish) (Arntzen and Teunis, 1993; Ildos and Ancona, 1994; Marnell, 1998; Oldham et al., 2000; Babik and Rafinski, 2001; Joly et al., 2001; Denoël et al., 2005; Skei et al., 2006; Denoël and Lehmann, 2006). Thus, newts in the Montenegrin karst area in many respects do not differ substantially from ones in the core of the species range in terms of breeding site characteristics.

Newts in the holokarst region are undoubtedly in great danger due to its inherent unpredictability. Thus, good connectivity between breeding habitats is essential if recolonization is to follow extinction. However, due to extremely hostile terrestrial habitats in the holokarst (dry terrains with rock/crevices, cliffs, screes, etc.) and great distances between ponds, corridors between water bodies are most likely to be ineffective. Highly relevant for conservation is the fact that European newts in general exhibit limited dispersal abilities and spawning-site fidelity and structuring as demes (see Smith and Green, 2005, for data). In the historic past, newts were favored, as an increasing number of spawning sites became available over time through creation of ponds for cattle drinking and human consumption within karstic ecosystems. But this trend is now in reverse. The number of amphibian breeding habitats in the studied karst regions has been steadily decreasing over the last decades. The ponds are often perceived as secondary habitats or are entirely managed by man. Many such ponds have been abandoned, which makes them less attractive for amphibians, especially for newts, as they undergo degradation and lose the ability to retain water, or else the forest has regenerated rapidly (personal observations and interviews with local people).

In summary, although each of the newt species has some specific habitat requirements, the studied water body characteristics were of low discriminative power to discern breeding sites that were or were not inhabited by a particular newt species. In other words, there are no certain "diagnostic" aquatic habitat characteristics for newt species occurrence in the Montenegrin karst region. Also, the results of this survey gave no indication that newts in the Montenegrin karst region have extended their ecological settings in any great measure. Furthermore, our results emphasize the importance of local processes in governing the incidence of newt species in the karst region of Montenegro.

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# КАРАКТЕРИСТИКЕ РЕПРОДУКТИВНИХ СТАНИШТА ЕВРОПСКИХ МРМОЉАКА (TRITURUS MACEDONICUS, LISSOTRITON VULGARIS И MESOTRITON ALPESTRIS, SALAMANDRIDAE) КАРСТА ЦРНЕ ГОРЕ

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Истраживано је присуство три врсте европских мрмољака у црногорском карсту, малог (Lissotriton vulgaris), источног главатог (Triturus macedonicus) и планинског мрмољка (Mesotriton alpestris), као и карактеристике њихових репродуктивних станишта. У погледу броја репродуктивних станишта, мали мрмољак је најчешћа врста, затим следи планински и на крају источни главати мрмољак. Истраживана водена станишта без мрмољака нису показала значајне разлике у карактеристикама у поређењу са воденим стаништима са мрмољцима. Фактори који најбо-

ње објашњавају уочену варијабилност карактеристика репродуктивних станишта мрмољака су тип и порекло станишта. Планински мрмољак примарно насељава језера природног порекла, док источни главати мрмољак насељава антропогена станишта (најчешће каменице и ублове). Мали мрмољак насељава различите типове станишта природног или антропогеног порекла. Особине репродуктивних станишта мрмољака Црне Горе не разликују се умногоме од особина репродуктивних станишта популација из центра ареала ових врста.