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Fluctuations in population abundance in two anurans from Central Serbia

Bogdan Jovanović[1], Jelka Crnobrnja-Isailović[1,2]

1 *Institute for Biological Research "Siniša Stanković", University of Belgrade, Despota Stefana Boulevard 142, 11060 Belgrade, Serbia*
2 *Faculty of Sciences and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia*

http://zoobank.org/CAB7B2FD-0385-4899-8D11-BF7A361BB812

Corresponding author: *Jelka Crnobrnja-Isailović* (jelka@pmf.ni.ac.rs)

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Abstract

We monitored the population size of the agile frog (*Rana dalmatina*) and the common toad (*Bufo bufo*) from 2011 or 2012, respectively, to the year 2017 at a syntopic breeding site in the vicinity of Belgrade. Adult *R. dalmatina* population size had minor fluctuations during the years of study (from 351 to 108 frogs). On the contrary, the adult *B. bufo* population was widely fluctuating towards decline (from 1158 to 141 toads). In both species, population fluctuations were not significantly related to variation of meteorological parameters (air temperature, humidity, precipitation). Density dependence effects on population size were not detected in either species. Apart from possible effects of climate change, the indicated trend towards decline of the monitored *B. bufo* population could also be the outcome of common population fluctuations or of increasing anthropogenic impact (vicinity of settlement and agricultural land). More years of monitoring more than one population are required to obtain precise information. Nevertheless, our results seem to be coherent with other studies that recommend conservation action for this species.

Key Words

Bufo bufo, climate variation, population growth rate, population size variation, *Rana dalmatina*, south-eastern Europe

Introduction

Many researchers all over the world report evidence on amphibian decline (Carey and Alexander 2003 and references therein). Recent estimations suggest that 41% of the all known amphibian species are threatened (IUCN Red List 2018). The causes of their decline are multiple, ranging from road kills through habitat loss and degradation, pollution, climate change, increased ultra-violet radiation, to the spread of infectious diseases (Carrier and Beebee 2003; Reading 2007; Bonardi et al. 2011; Petrovan and Schmidt 2016). Many of these factors are connected and therefore have joint effects (Bonardi et al. 2011). Araújo et al. (2006) suggested that the highest amphibian decline in Europe will occur in the Iberian Peninsula, southern France, Italy and eastern Europe, while some increases in amphibian abundance are projected for northern parts of central Europe. The same authors predicted the loss of amphibian species in areas where climate is changing towards an increase in ambient temperature and a decrease in humidity.

Monitoring is a key activity for establishing amphibian population trends and planning conservation actions. However, monitoring actions are more typically carried out on rare, endangered species within protected areas rather than on common species from human-altered habitats and which can lead to the extinction of local populations before any action is taken (see in Petrovan and Schmidt 2016). This is a good argument for an increase in monitoring efforts focused on common species and here we report the results of six years' monitoring of one local population of *Rana dalmatina* or the agile frog (2011–2017) and seven years' monitoring of syntopic *Bufo bufo* or European common toad population (2010–2017).

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Rana dalmatina is widespread in Southern Europe. It is classified as "Least Concern" by the IUCN (Kaya et al. 2009), included in both the Habitats Directive (annex IV animal and plant species of community interest in need of strict protection) and the Bern convention (appendix II: strictly protected fauna species). *Bufo bufo* is one of the most widespread amphibian species in Europe (Sillero et al. 2014), is also proclaimed as "Least Concern" by the IUCN (Agasyan et al. 2009) and is included in the Bern convention (appendix III: protected fauna species). So far, monitoring initiatives for *R. dalmatina* have been conducted in Greece (Sofianidou and Kyriakopoulou-Sklavounou 1983), Romania (Hartel 2008a; Hartel 2008b), Austria (Waringer-Löschenkohl 1991), France (Combes et al. 2018), Sweden (Strömberg 1988) and Hungary (Puky et al. 2006). *Bufo bufo* has been monitored in the UK (Cooke 1972; Reading and Clarke 1995; Carrier and Beebee 2003; Reading 2007), Germany (Wagner 2013), Switzerland (Petrovan and Schmidt 2016) and Austria (Kyek et al. 2017).

Both species are widespread in Serbia and are strictly protected by national legislation (regulation on the proclamation and protection of strictly protected and protected wild species of plants, animals and fungi – appendix I, Anonymous 2010), but were not included in the national Red Book of threatened amphibians (Kalezić et al. 2015). The main objectives of this study were to (i) present preliminary results on population fluctuations of the two species and (ii) to relate the population fluctuations to meteorological variables. To our knowledge, an amphibian monitoring initiative is new for Serbia and the information presented in this paper is the first outcome of a long-term monitoring project.

Methods

Study site

The study site is a permanent pond on the outskirts of Belgrade (Zuce village, 44°40.93'N, 20°33.12'E, altitude 240 m) in Central Serbia (Tomašević et al. 2008). The pond was created by humans some 50 years ago. There are two permanent streams that bring the water in and one stream that exits the pond; in summer, the exit stream is sometimes dry. The pond is elongated, approximately 70 m long and 35 m wide or 2420 m^2 in overall area. The maximal depth is around two metres. The pond is located in a deciduous oak forest patch: a thermophilic oak community *Quercetum frainetto-cerris* (Ćorović and Crnobrnja-Isailović 2018). Blackberry shrubs are present around parts of the pond and many *Typha* sp. occur in some parts of the littoral zone. Small fish are abundant in the pond and were originally introduced by local sports fishermen shortly after the pond was made. According to local inhabitants, more fish species have continuously been introduced over the years (some cyprinids and many brown bullheads, *Ameiurus nebulosus*). The following amphibian species were repeatedly recorded: fire salamander (*Salamandra salamandra*), *B. bufo*, *R. dalmatina* and water frogs of the *Pelophylax esculentus* complex. Fire-bellied toads (*Bombina variegata*) and tree frogs (*Hyla arborea*) were spotted only occasionally during the monitoring period. Human activity has been occurring in the form of frequent visits by locals and fishermen, sometimes by trucks and tractors just 15 m away from the pond. The closest household is situated 300 m from the pond and the closest main country asphalt road is 530 m away (very few amphibian corpses were spotted on this road, so migrations probably follow a path that does not dissect the road). The closest major agricultural land is 590 m away. Traces of possible chemical pollution were sporadically observed in the form of white foamy strings and an oily covering in some parts of the pond. There is a permanent occurrence of trash, either submerged or lying around the banks of the pond.

Field procedures

We analysed data starting from 2012 to 2017 and from 2011 to 2017 for the *R. dalmatina* and *B. bufo*, respectively. We made several visits from 2011–2015 to the site from early March onwards. From 2015, visits became more frequent e.g. on a weekly level, starting in January or February. For *R. dalmatina,* spawning begins one or two weeks before the mating period of *B. bufo* and ends within this period. We counted egg clutches of *R. dalmatina* and adults of both species regularly throughout the breeding season and the number of counting days varied from 2 to 20 (10.4 on average). On each visit, we inspected the pond and recorded anuran presence by walking slowly along the bank, always in a clockwise direction from a fixed starting point. From 2015, for *R. dalmatina,* we counted the number of egg clutches per day until all had hatched, while for the *B. bufo*, we performed regular daily scanning of reproductive activity when the number of amplexuses seen in the pond reached 100. Visits became less frequent when the number of toads started to decrease significantly but were not stopped until the last adult toad left the pond. We searched for adults and egg clutches across the whole surface of the pond, but found the vast majority of both near the pond edge.

We collected meteorological data used in this study from the Republic Hydrometeorological Institute of Serbia (http://www.hidmet.gov.rs) with an included period from 1 December to 28 or 29 February, except data on mean daily precipitation for the period 01.01.2015 – 19.01.2015 which are missing. In the raw database, the mean daily humidity was sometimes slightly higher than 100%, but we treated all those records in our analyses as 100%.

Statistical analyses

For *R. dalmatina*, we estimated an annual adult population size (*N*) as the maximum number of egg clutches counted during the reproductive season of a particular year following Piha et al. (2007) and Hartel et al. (2009). They used number of egg clutches as a proxy to estimate population size (*N*). For *B. bufo*, we considered the maximum daily number of reproducing individuals counted during one reproductive season to be the annual adult population size (*N*) for that year. Although some *B. bufo* individuals do not reproduce each year (Loman and Madsen 2010), those that migrate towards the ponds can be counted as reproductive. For every year of study, we also determined the annual sex ratio as the ratio of the maximum daily number of adult males to the maximum daily number of adult females recorded in the pond (Lodé 2009).

The set of meteorological data included annual average (AVG), minimum (MIN) and maximum (MAX) values of mean daily temperature (T, °C), humidity (H, %) and precipitation (P, mm) for three winter months (December, January and February) for each year (set: AVGT, AVGH, AVGP, MINT, MINH, MINP, MAXT, MAXH, MAXP). We chose meteorological data from that part of the year for comparison with the fluctuation of population size in the two analysed species because winter time is very important for anuran survival in the temperate climate zone: frogs and toads spend those months in hibernation and the quality of hibernation is reflected in the body condition of post-hibernating individuals (see in Reading and Clarke 1995 and Reading 2007).

We also made an estimate of population growth rate for both species following Houlahan et al. (2000) and Hartel (2008a), where $\Delta N = \log (N + 1)_{t+1} - \log (N + 1)_t$ and *N* is an adult population size in a certain year (t). We applied non-parametric statistics to test for trends in population size over the years of monitoring because datasets for both species were small (six and seven years of monitoring for the *R. dalmatina* and *B. bufo*, respectively). To provide a glimpse into the mechanisms of the population fluctuations, we tested population size variation for density dependence by using Kendall rank correlation (comparing estimated adult population size with the population growth rate).

Results

The number of *R. dalmatina* egg clutches varied from 351 in year 2012 to 108 in year 2015 (Table 1). The population growth rate (the yearly rate of change in the maximal recorded number of egg clutches) was lowest between 2016 and 2017 (-0.76) and highest between 2015 and 2016 (0.91) with a negative mean value of -0.2 for the whole period (Table 1). We did not confirm the density dependence effect on population size for this population (Kendall Tau = -0.60, Z = -1.47, p = 0.14) (Fig. 1). The population size did not show a trend of change over the six-year period (Kendall Tau = -0.33, Z = -0.94, p = 0.35).

In the analysed *B. bufo* population, the total number of adult individuals, as well as the number of adult males, seemed to be declining over the monitoring years (Table 2), although intersected by a few increases (Kendall Tau = -0.62, Z = -1.95, p = 0.05 for population size). We recorded most individuals in the year 2012 (1158 adult individuals, of which 1034 were males), while the lowest number we noted in 2016 (only 141 adult individuals, of which 139 were males) (Fig. 1). Additionally, the number of reproductive females was very low compared with the overall number of reproductive males in every year of the monitoring period. In 2017, the operational sex ratio even achieved the value of 44:1. Population growth

Table 1. Population parameters, meteorological parameters, and descriptive statistics of variables for *Rana dalmatina* population.

Year	Population parameters		Meteorological parameters								
	N	Δ*N*	Temperature °C			Humidity %			Precipitation mm		
			AVG	MIN	MAX	AVG	MIN	MAX	AVG	MIN	MAX
2012	351	-0.32	1.66	-12.10	14.90	76.01	52.10	98.00	2.16	0.00	36.80
2013	254	-0.71	3.25	-4.80	13.30	78.31	47.80	94.90	2.06	0.00	18.50
2014	124	-0.14	5.36	-5.90	16.90	75.62	45.00	98.20	0.58	0.00	8.90
2015	108	0.91	4.17	-9.20	11.20	76.94	49.60	97.60	2.01	0.00	19.00
2016	271	-0.76	4.95	-9.20	15.00	79.44	38.00	100.00	1.03	0.00	12.80
2017	126		0.42	-13.10	16.40	76.50	31.00	100.00	0.66	0.00	17.60
Descriptive statistics											
Valid Number of years	6	5	6	6	6	6	6	6	6	6	6
Mean	206	-0.20	3.30	-9.05	14.62	77.14	43.92	98.12	1.42	0.00	18.93
MIN	108	-0.76	0.42	-13.10	11.20	75.62	31.00	94.90	0.58	0.00	8.90
MAX	351	0.91	5.36	-4.80	16.90	79.44	52.10	100.00	2.16	0.00	36.80
SD	100.28	0.68	1.94	3.28	2.10	1.46	7.97	1.88	0.74	0.00	9.59

N = maximum number of egg clutches (population size); Δ*N* = changes in egg clutches number (population growth rate); AVG = average value; MIN = minimum value; MAX = maximum value; SD = standard deviation.

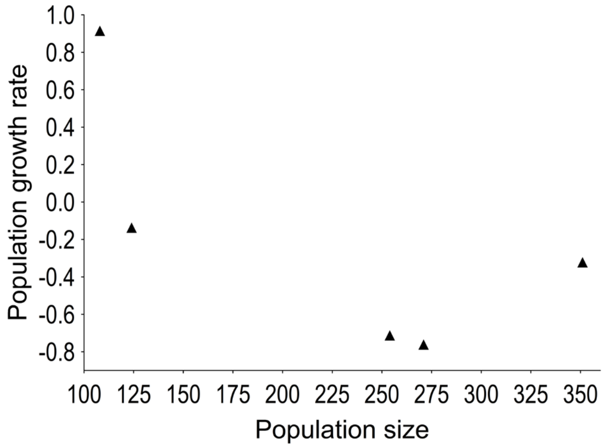

Figure 1. Variation of *R. dalmatina* population growth rate with population size over five consecutive years.

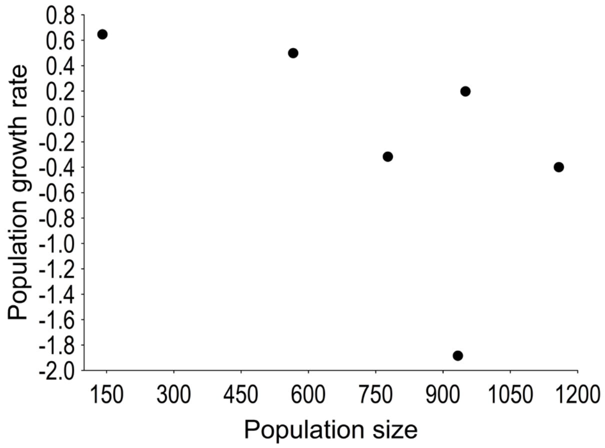

Figure 2. Variation of *B. bufo* population growth rate with population size over six consecutive years

Table 2. Population parameters, meteorological parameters, and descriptive statistics of variables for *Bufo bufo* population.

Year	Population parameters					Meteorological parameters								
						Temperature °C			Humidity %			Precipitation mm		
	N	Nm_{max}	Nf_{max}	SR	ΔN	AVG	MIN	MAX	AVG	MIN	MAX	AVG	MIN	MAX
2011	950	885	65	14:1	0.20	1.69	-8.10	16.60	80.01	56.30	95.90	1.83	0.00	27.20
2012	1158	1034	124	8:1	-0.40	1.66	-12.10	14.90	76.01	52.10	98.00	2.16	0.00	36.80
2013	777	706	170	4:1	-0.32	3.25	-4.80	13.30	78.31	47.80	94.90	2.06	0.00	18.50
2014	566	551	59	9:1	0.50	5.36	-5.90	16.90	75.62	45.00	98.20	0.58	0.00	8.90
2015	933	871	62	14:1	-1.88	4.17	-9.20	11.20	76.94	49.60	97.60	2.01	0.00	19.00
2016	141	139	8	17:1	0.65	4.95	-9.20	15.00	79.44	38.00	100.00	1.03	0.00	12.80
2017	270	264	6	44:1		0.42	-13.10	16.40	76.50	31.00	100.00	0.66	0.00	17.60
Descriptive statistics														
Valid number of years	7	7	7	7	6	7	7	7	7	7	7	7	7	7
Mean	685	636	71	16:1	-0.21	3.07	-8.91	14.90	77.55	45.69	97.80	1.48	0.00	20.11
MIN	141	139	6	4:1	-1.88	0.42	-13.10	11.20	75.62	31	94.90	0.58	0.00	8.90
MAX	1158	1034	170	44:1	0.65	5.36	-4.80	16.90	80.01	56.30	100	2.16	0.00	36.80
SD	375.55	334.95	59.25	13.23	0.92	1.87	3.01	2.06	1.72	8.65	1.91	0.69	0.00	9.29

N = maximum daily number of reproductive individuals (census population size); Nm_{max} = maximal daily number of observed males during reproductive season; Nf_{max} = maximal daily number of observed females during reproductive season; SR = sex ratio (♂:♀); ΔN = population growth rate; AVG = average value; MIN = minimum value; MAX = maximum value; SD = standard deviation.

rates ranged from –1.88 between 2015 and 2016 to 0.65 between 2016 and 2017 and were negative for half of the analysed years. The mean population growth rate for the whole monitoring period was negative (-0.21) (Table 2). We also did not confirm density dependence for this species (Kendall Tau = -0.60, Z = -1.69, p = 0.09) (Fig. 2).

Discussion

The population of *R. dalmatina,* analysed in this study, can be considered as stable during the monitoring period of six years, despite minor fluctuations in size. On the contrary, the size of the syntopic *B. bufo* population apparently varied between consecutive years. Fluctuation patterns of this population size suggested its decline. This *B. bufo* population might be threatened by extinction as the number of adult members (especially females) was very low in the last two years of the study.

Literature data revealed a variety of information on numbers of egg clutches in *R. dalmatina*: they varied from 6 to 82 (average of 26) and from 7 to 68 (average of 37) at two breeding sites in Austria (Waringer-Löschenkohl 1991, seven years of monitoring), from 60 to 133 (average of 97) in Sweden (Strömberg 1988, seven years of monitoring), from 60 to 171 (average of 103) in Austria (Gollmann et al. 1999, four years of monitoring), from 198 to 980 in Greece (Sofianidou and Kyriakopoulou-Sklavounou 1983, three years of monitoring) and from 265 to 581 (average of 410) in Romania (Hartel 2008a). Longer monitoring is necessary to obtain a more precise estimation of the population trend over the years, although both Hartel (2008b) in Romania and Combes et al. (2018) in France found no evidence of decline in egg clutches abundance of *R. dalmatina* populations monitored for 11 years and 29 years, respectively. However, some localised demographic (Kaya et al. 2009) and population genetic diversity (Lesbarrères et al. 2003 for France) declines have been reported for this species.

Literature data show that *B. bufo* population size estimates varied from 238 to 614 females and from 532 to 1045 males in Sweden (Loman and Madsen 2010, 5 years of monitoring), from 2715 to 3629 individuals in England (Reading and Clarke 1995, 7 years of monitoring), from 38 (averages of counted individuals over 2 years of monitoring) to 1832 (averages of counted individuals over 5 years of monitoring) of individuals in Germany (Wagner 2013) and from 4 (mean number of individuals per year over 4 years of monitoring) to 8581 (mean number of individuals per year over 10 years of monitoring) individuals in Austria (Kyek et al. 2017). The decline of *B. bufo* is already recorded in literature, where some causes of decline are attributed to road mortality, climate change, habitat loss and degradation and infectious diseases (Carrier and Beebee 2003; Reading 2007; Bonardi et al. 2011; Petrovan and Schmidt 2016). For example, Cooke (1972) reported the decrease of *B. bufo* abundance in the UK during the 1960s, suggesting habitat loss as the cause. Bonardi and Ficetola (2012) reported a decline in 21 out of 30 *B. bufo* populations during 15 years of monitoring in Italy, suggesting road mortality as the major cause of decline. Petrovan and Schmidt (2016) analysed volunteer data on *B. bufo* populations in the UK and Switzerland (29 and 39 years of monitoring, respectively) and they reported declines in both countries, again referring to roads as the factor with a great negative impact on *B. bufo* populations (both countries have dense road systems and intense habitat fragmentation). Popescu et al. (2013) conducted research in Romania and their scenarios of climate change predicted a severe decrease in abundance of the *B. bufo* in the next 40 years due to possible future climate changes. In contrast, Bonardi and Ficetola (2012) recognised a positive trend in three of 30 *B. bufo* populations monitored for 15 years in Italy, Kyek et al. (2017) reported stability of populations situated close to the road network in Austrian Alps during 21 years of monitoring and Wagner (2013) also claimed that most of the *B. bufo* populations in Germany were stable.

The two syntopic anuran populations from this study are situated in the vicinity of Belgrade, the capital of Serbia. Continuous urbanisation of the city has also been affecting villages close to this amphibian breeding place by causing inevitable fragmentation and pristine habitat loss over time (see in Vegetation Continuous Fields – VCF, Sexton et al. 2013). However, Ponsero and Joly (1998) suggested that the open environment around the pond should be more suitable for tadpole development than shady forest with a large quantity of decomposing organic material (hypoxia and low productivity). The other possible reason for *B. bufo* population decline could be intensification of deteriorating human activities (inadequate waste disposal possibly including hazardous chemicals from nearby agricultural fields, forest exploitation and increased predation on toads during their migrations); however, so far, we could not obtain clear evidence. Finally, the observed declining trend could also reflect common population fluctuations.

Another possible cause of the *B. bufo* population decline could be climate change but our data include too short a period in time for testing relationships between changes in population size and meteorological data. Literature data provide a number of examples on how autumn and winter temperature affect amphibians, from positive effects of higher temperatures on the agile frog (Combes et al. 2018) to negative ones for *B. bufo* (Reading and Clarke 1995; Reading 2007). The positive influence of autumn and winter precipitation on amphibian populations was documented in a few research projects (Jensen et al. 2003; Hartel 2008a; Cayuela et al. 2014; Benard 2015; Salvidio et al. 2016; Combes et al. 2018) and higher humidity should decrease desiccation risks during hibernation (Combes et al. 2018). In this study, we revealed a trend towards an increase in winter humidity over the years of monitoring, while temperature variation patterns seemed random. However, discussion about the influence of temporal change on the *B. bufo* population could not be undertaken without more years of research and inclusion of more populations into the study.

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