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Altered breeding behaviour in some amphibians from an artificial habitat in the Jiu Gorge National Park, Romania

Severus-Daniel Covaciu-Marcov[1,*], and Felicia-Nicoleta Sucea[2]

In Romania, the number of amphibian species in the Carpathian Mountains is higher than in any other regions in the country, particularly the plains (Cogălniceanu et al., 2013). Nevertheless, in some areas of the southern Carpathians, the very steep slopes limit the formation of the aquatic habitats necessary for amphibian reproduction, a fact reflected in their distribution (Covaciu-Marcov et al., 2009a, b, 2020). Such a region is Jiu Gorge National Park (JGNP) in the southwestern part of the southern Carpathians, where a remarkable amphibian diversity is characterized by species with small population sizes or rare occurrences due to the limitations of aquatic habitat availability (Covaciu-Marcov et al., 2009a). In this area, artificial habitats appear to be an important resource for amphibians (Ile and Sucea, 2018). Thus, we initiated studies on the amphibian fauna in artificial habitats at a disused stone quarry in JGNP. There, we identified some amphibian species rare in JGNP and some changes in their reproductive behaviour. Because such cases were not reported in previous studies (e.g., Dobre et al., 2007, 2009; Covaciu-Marcov et al., 2009a; Sucea et al., 2014; Ile and Sucea, 2018), we do so herein.

In Spring 2019 in JGNP (45.2178°N, 23.3768°E, elevation 358 m), we observed amplexus between a *Bufo bufo* (Linnaeus, 1758) female and a *Bufotes viridis* (Laurenti, 1768) male, and a double amplexus between two *B. bufo* males and a single female of the same species. The first case was identified on 20 April 2019, and the second on 18 May 2019. Both were recorded in a disused stone quarry at Meri, in an area where the stone had been crushed and loaded into trucks. After abandonment of the quarry, approximately ten puddles formed, with diameters of 1–6 m² and a maximum depth of 30–40 cm. These pools are devoid of vegetation and have a substratum made from small stones (Fig. 1). In these puddles, we encountered *Lissotriton vulgaris*, *Triturus cristatus*, *Bombina variegata*, *Bufo bufo*, *Bufotes viridis*, *Hyla arborea*, *Pelophylax ridibundus*, and *Rana dalmatina*, all of which had previously been recorded in JGNP (Covaciu-Marcov et al., 2009a). At a distance of 50 m away from these puddles, the former settling pond of the quarry is known as a habitat for newts (Ile and Sucea, 2018). The heterospecific amplexus was observed in a larger nearby puddle, where another five *B. bufo* and spawn were present. The heterospecific pair remained motionless in the shallow water and could easily be photographed (Fig. 2). The *B. bufo* double amplexus was observed in one of the smallest puddles. One of the males was in axillary amplexus, the normal position for *B. bufo* (Fuhn, 1960), and the other was in abnormal, inguinal amplexus (Fig. 3).

Although rare, unusual amplexi have repeatedly been reported in the last years in different species, regions, and combinations (e.g., Mollov et al., 2010; Gül et al., 2018; Lirio et al., 2019; Ayres and Dominguez-Costas, 2020; Gama et al., 2020; Shin et al., 2020). Thus, the presence of the two unusual amplexi is not unexpected, and in Romania such situations have already been reported in *B. bufo* (Hartel et al., 2007; Gherghel et al., 2008; Strugariu and Gherghel, 2008; Mačát et al., 2019). What is unexpected is the fact that this phenomenon was recorded one year after we completed a 10-year study of the amphibians in JGNP and never observed it (e.g., Dobre et al., 2007, 2009; Covaciu-Marcov et al., 2009a; Sucea et al., 2014; Ile and Sucea, 2018). The most probable explanation is that the artificial habitats, which bring together more individuals from different species in a relatively limited area, create diverse assemblages where mistaken identities among hormone-boosted individuals produce aberrant behaviours. In addition, reproductive agglomerations of other species in artificial conditions seem to lead to atypical amplexus (e.g., Shin et al., 2020).

In JGNP, *B. bufo* was considered a rare species in the past, with few distribution records and individuals,

[1] Department of Biology, Faculty of Informatics and Sciences, University of Oradea, 1 Universităţii, Oradea 410087, Romania.
[2] Jiu Gorge National Park, Strada Strada Locotenent Colonel Dumitru Petrescu 3, Târgu-Jiu 210182, Romania.
[*] Corresponding author. E-mail: severcovaciu1@gmail.com

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Figure 1. The habitat at Meri Quarry, Jiu Gorge National Park, Romania. Photo by Felicia-Nicoleta Sucea.

Figure 2. Heterospecific amplexus of a *Bufo bufo* female with a *Bufotes viridis* male at Meri Quarry, Jiu Gorge National Park, Romania. Photo by Severus-Daniel Covaciu-Marcov.

while *B. viridis* was more common (Covaciu-Marcov et al., 2009a). The data from the quarry shows that *B. bufo* is now better represented. Nevertheless, 11–12 years ago (Covaciu-Marcov et al., 2009a) there were no frogs in the quarry and the puddles were crossed daily by numerous trucks and other machinery, with the water being polluted by stone suspensions. After the closure of the quarry, the few amphibians still surviving in the region started to reproduce in the newly created artificial habitats and managed to increase their populations, as was the case with newts (Ile and Sucea, 2018). Thus, it appears that artificial habitats represent an opportunity for amphibians. At the same time, gathering a lot of individuals in small, artificial spaces appears to provide opportunities for altered reproductive behaviour. The habitats are small, shallow, with clear water and without shelters, and contact between individuals is a game of chance. However, with a low water level habitats are vulnerable to drought, thus the amphibians are under a strong pressure to reproduce. Moreover, these pools lack vegetation, which ordinarily is important for *B. bufo* spawns (Loman and Madesen, 1986).

Bufo bufo is normally an explosive breeder with individuals present at reproductive sites for only a few days in early spring (Wells, 1977; Hartel et al., 2007). For example, in Sweden, *B. bufo* reproduction lasted only seven days (Loman and Madsen, 1986). In contrast, the reproduction of the species extended over at least two months (March–May) in the Meri quarry. While we cannot know whether the same individuals were in the water each time, the species is expected to leave the water much sooner, as *B. bufo* normally reproduce in Romania between the end of March and the beginning of April (Fuhn, 1960). This alteration of *B. bufo* reproductive behaviour may facilitate the appearance of heterospecific amplexus because *B. viridis* prefers higher temperatures (Fuhn, 1960). In Romania, *B. viridis* normally reproduces later than *B. bufo*, from April–June, and stays in the water longer compared with *B. bufo* (Fuhn, 1960). Thus, these two species do not usually meet during their reproductive activities. At Meri, *B. viridis* was not present in the water before what is normal for in Romania, but *B. bufo* stayed longer than normal (Fuhn, 1960). In the same year, in other areas in JGNP, *B. bufo* was present in the water for reproduction only in March. Thus, this phenomenon does not appear to be related to the JGNP as a locality, but to the quarry and its artificial pools in particular. Most likely, the artificial habitat modified the reproductive behaviour. The shallow puddles, with stony substratum, dried out frequently in the spring and fragmented the breeding season, and each time when they carried water, frogs re-entered them. In April and May we observed spawns, and this shows that the species reproduced well beyond its normal breeding period in Romania (Fuhn, 1960). Thus, the best explanation for the frogs' adjusted reproductive behaviour is the artificial habitat. It offers opportunities

Figure 3. Double amplexus in *Bufo bufo*, showing one female with two males. Photo by Severus-Daniel Covaciu-Marcov.

not found naturally for amphibians in the region that may help increase numbers of a population, but at the same time it may lead to modified reproductive behaviour.

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