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Impacts of resource limitations on the reproduction behaviour in the Agile Frog (*Rana dalmatina*) on the territory of Natura park "Shumensko plato"

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Abstract. The Agile Frog (*Rana dalmatina*) is one of the anurans species inhabiting the Nature Park "Shumensko Plato" (north-eastern Bulgaria). In 2019 and 2020 we studied the general ecology of the population in the Park and in particular the breeding activity of the frogs. During our survey we registered extension of the breeding season and we also report on the longest amplexus for *R. dalmatina* - 12 days (detected in an artificial water basin).

Key words: Key words: amplexus, breeding activity, artificial ponds.

Introduction

The Agile Frog (*Rana dalmatina* Fitzinger in Bonaparte, 1838) is a terrestrial ranid species that is widely distributed in Europe, hence these anurans do not form dense populations (Naumov 1999). On the Balkan-peninsula the adults of *R. dalmatina* emerged from hibernation in late February to early March. In this species, the breeding period starts immediately after hibernation (Stojanov *et al.* 2011, Iosob & Prisecaru 2014). The amplexus in these frogs is of pectoral type and benefits to the cloacal alignment and thereby helping successful fertilization (Mollov et al. 2010). In *R. dalmatina* the larger males are able to hold the females in amplexus for longer periods of time, but the size of the female is not definitive for the length of the amplexus (Vági & Hettyey 2016). In the present study we report on some peculiarities in the breeding behaviour of the Agile Frogs from the Nature park "Shumensko plato".

Material and Methods

In this survey we studied the breeding activity of the Agile Frog on the territory of the Nature Park "Shumensko Plato". We mapped all water basins on the investigated territory by using hand held GPS system Garmin Etrex 30 (Garmin International Inc., Kansas, USA). In a total area of 3930,7 hectares we detected only 4 water basins which retain water volumes for prolonged time. These basins are grouped two by two in two sites with the following coordinates: N43.248888; E26.893333 and N43.248888; E26.892777 (Datum: WGS84). They are located in habitat of high conservation priority (European Directive 92/43/EEC). According to the Palearctic habitat classification of Moss & Davies (2002) theses habitats may be classified as type 41.2B Pannonic oak-hornbeam forests.

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We provided daily direct observation during the whole breeding period of the Agile Frogs. To determine the total duration of the spawning period, our visits to the study area started in mid-February and ended in the middle of May. In the winter of 2019 we set up a small artificial pond with dimensions 50 x 25 x 20 cm to gain information on the breeding behaviour of limited number of frogs which may be identified and documented daily. We used Nikon D7000 for photography and photo trap Ltl Acorn 5210M for predator detection.

Results and Discussion

In the Agile Frogs, the males are the first to arrive at the breeding sites (Hettyey *et al.* 2005, Lode & Lesbarreres 2004, Pavignano *et al.* 1990). In 2019 a male *Rana dalmatina* appeared in the studied ponds on 06.03 and in 2020, the first frog appeared earlier due to the warmer weather – on 25.02.

The duration of the breeding season in *R. dalmatina* lasts for of about 30 days (Biaggini *et al.* 2018, Combes et al. 2018, Hettyey et al. 2005, Vági & Hettyey, 2016). In the investigated ponds, the first egg-clutch was detected on 06.03 in 2019 and on 28.02 in 2020. In both years, new egg-clutch were still appearing until the end of April. This indicates on extraordinary prolonged breeding seasons in the investigated population (see Stojanov *et al.* 2011). For the Agile Frogs Ward & Griffiths (2015) reported a prolonged spawning period of six to seven weeks. These authors propose that the increase in the population had caused the extend in the breeding time. The population of *R. dalmatina* on the territory of the Natura park "Shumensko plato" is apparently not dense and the reason for the prolonged breeding season have to be related with the limitation in the number of the ponds suitable for spawning. Naumov (1999) reports that the Agile Frogs overwinter in the vicinity of the water basins they use for reproduction. Presumably, in our case, the specimen which are hibernating besides the water basins that normally forms after winters with high snow and rain falls, had to migrate for prolonged time to reach the only two water basins available after the dry winters of 2019 and 2020. The patterns of the road mortality of the Agile Frogs in the vicinity of the ponds strongly support that hypothesis. In the first morning with detected frog activity we found 20 killed frogs on a 30 m long road section in 2019 and 30 more were found in the same circumstances in 2020. After the initial period of the activity, more road kills were detected, but they were only sporicidal and scattered on wider section of the road. A possible explanation can be related to an initial mass migration on a narrow front of the "local" male frogs overwintering in the vicinity of the ponds and migrating simultaneously in the first night of activity.

During the prolonged breading seasons, around 20 egg-clutches were registered out of the water near the ponds every year (Fig. 1). In 2019 the last such case was observed on 11.05 and in 2020 on 12.05. The presence of females carrying eggs so late in the season is a strong indication of a prolonged reproduction season. It is possible that these females migrate from far destinations toward the few water basins suitable for spawning. Our camera trap revealed that such female frogs were attacked by day active birds of prey (*Buteo buteo, B. rufinus, Aquila pennata*). Selas et al. (2007) and Swan (2011) reported that some frogs are regular part of the common buzzard's diet. In the investigated region of the park lives a resident pair of common buzzards and 87% of the attacks were performed by these birds. By consuming of the frogs, the birds removed the eggs.

On 08.03.2019 a *R. dalmatina* male occupied the small artificial basin that we had prepared in the winter. On 14.03.2019 two specimens (male and female) were observed in amplexus (Fig. 2). On the base of the photo documentation, we were able to identify these specimens in the next days. The male had released the female after the egg deposition on the 12-th day of the amplexus (25.03.2019). For *R. dalmatina* was reported that the male may not be able to release the female until the spawning is complete (Hettyey *et al.* 2005). These authors report that the amplexus in the Agile Frog can extend from several hours up

to 4 days. In 2020 a male Agile Frog occupied the artificial pond on 28.02. This specimen was detected inside the basin until 10.03. During this period, the presence of a female was not registered, but on 11.03 an egg-clutch was found. On 30.03.2020 another male was registered inside the basin. However, no new egg-clutches was observed. The larvae from the only egg-clutch in the artificial pond hatched on 14.04 (after 34 days). In 2019 the larvae needed 31 days to hatch. These results confirmed the data known from Bulgaria according to which the average duration of the egg phase is 30 days (Naumov 1999).

Fig. 1. Registered egg-clutches out of the water near the ponds.

Fig. 2. Male and female *Rana dalmatina* in an artificial basin in amplexus for 12 days.

Miaud & Merilä (2001) explain the variation of the beginning of reproduction among populations as a result of adaptation of the local populations to specifics in the environments. The feeding activity before hibernation may also impact the breeding behaviour (Elmberg & Lundberg 1991). Such factors may limit the reserves of the frogs during the hibernation, and may impact the reproduction activity (Hartel 2005). In the case of our study, the aberrations in the breeding behaviour of the local population can be explained by the limitation of the resources in form of suitable spawning basins in the region. The prolonged duration of the breeding season, the extremely long amplexus time

and the presence of many egg clutches out of the water in the middle of May reflects the reaction of the Agile Frogs on the meteorological and hydrological conditions in 2019 and 2020.

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