# HABITAT DISTRIBUTION AND VOCAL ACTIVITY OF THE MARSH FROG (*PELOPHYLAX RIDIBUNDUS*), THE EUROPEAN GREEN TOAD (*PSEUDEPIDALEA VIRIDIS*) AND THE EASTERN SPADEFOOT (*PELOBATES SYRIACUS*) (ANURA: RANIDAE, BUFONIDAE, PELOBATIDAE) IN THE PROTECTED AREA OF RUPITE

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## ABSTRACT

The habitat distribution and vocal activity of three anuran species were studied in three localities in the protected area of Rupite. The heavy-built terrestrial species P. viridis and P.syriacus were found only in ponds, while the semi-aquatic P. ridibundus also occupied flowing waters. We observed increased nocturnal activity in the area around the thermal springs. This probably was due to the higher and relatively constant water temperature. We did not observe any activity of P.syriacus, however, in one pond we found numerous dead specimens. The reasons for the observed ecological and behavioral characteristics are discussed.

***Key words:*** *anurans, habitat distribution, vocalization, temperature, pH*

## INTRODUCTION

Nearly every aspect of amphibian physiology and behavior is affected by temperature. While locomotion in some species is relatively temperature independent over a narrow range of temperatures [13], aerobic metabolism is temperature dependent in all amphibians and generally increases in a linear fashion as temperature increases [16]. Body temperature is close to that of the immediate surroundings, especially the substrate. The amount of metabolic heat produced is so small that it is lost to the environment [8]. In warm environments most amphibian behavior seems to be associated with maintaining low body temperatures, but cryptic and nocturnal is likely a response to problems of water economy than to temperature [7]. Amphibians as a group have a wide range of thermal tolerances. However, preferred body temperatures usually are nearer the upper than the lower extremes of temperatures tolerated [3, 4]. By habits of postmetamorphic stages outside the breeding season [11] divides the amphibian species into terrestrial and semi-aquatic.

Despite the existence of some papers [1] the scientific data on the ecology of Bulgarian amphibians is still insufficient. This research aimed to establish the level of activity and the character of habitat distribution of three anuran species - the marsh frog *Pelophylax ridibundus* (Pallas 1771), the eastern spadefoot *Pelobates syriacus* (Boettger 1889) and the European green toad *Pseudepidalea viridis* (Laurenti 1768) - in the beginning of their breeding seasons in an area near the village of Rupite. All three are included in the Biodiversity Act – the marsh frog in appendix IV and the green toad and the eastern spadefoot in app. III, and the BERN convention – the marsh frog in app. III, the green toad and the eastern spadefoot in app. II. The green toad and the eastern spadefoot are terrestrial species with cryptic/nocturnal activity, while the marsh frog is a semi-aquatic species with twenty-four-hour activity [2]. Due to the fact that no terrestrial species lays its eggs in ponds where the marsh frog is by all means absent, the former two species could come in contact with the marsh frog during the breeding season, when they enter ponds to lay their eggs [1]. The chosen area included both ponds and slow-flowing canals formed by thermal springs. We expected a higher level of activity near the thermal springs compared to other localities.

## MATERIALS AND METHODS

The research was conducted on two separate occasions during the period 20$^{th}$ to 22$^{nd}$ of March and 17$^{th}$ to 20$^{th}$ of April 2011 in the protected area of Rupite. It is situated approximately 12km

from the city of Petrich, on the land of the village of the same name, on the right bank of Struma river, at the foot of the volcanic hill of Kozhuh (281m). The climate is very specific, transitional between continental and Mediterranean and characterized by hot dry summers and mild winters. We carried our research in three localities. Locality 1 (N 41º27'314", E 23°15'485" alt. 88m) included the thermal springs and the ponds and canal formed by them. The hot springs issuing in the area reach a temperature of 71-78°C and have an average discharge of 35 l/sec. Several small ponds (the largest with surface area about 0.02 ha) with varying temperatures (from over 60°C to 20°C) are formed near the springs and drain into a canal. Locality 2 (N 41°27'681" E 23°14'551" alt. 114m) was a medium-sized pond on the western side of the Kozhuh hill, near the road to Petrich. It had an approximate surface area of 2.68 ha. Locality 3 (N 41º27'277", E 23º14'559" alt. 119m) is south of locality 2, near a pumping station. It was the largest pond with surface area approximately 4.71 ha. Around localities 2 and 3 as well as around the canal draining the thermal springs there is abundant growth of reed (*Phragmites communis*).

The observations were carried out two times per day in the midday (14-16h) and evening (22-24h) hours for periods of two hours. We measured the air (up to 50 cm) and water temperatures of the localities, the respective hardness (pH) and carbonate hardness (kH), atmospheric pressure and vocal activity (when observed). The temperatures were measured using a digital water-resistant thermometer. The measurements were recorded with an accuracy of one-tenth of the degree on every observation, including the cases in which activity was not observed. Only the temperature of the microhabitat was measured since it has the greatest influence on the amphibian activity. For measuring the pH-value and the carbonate hardness of the water we used the Sera GmbH pH-test and kH-test, which have an accuracy of 0.5 and 1 degrees respectively. Samples were taken once at every locality. The atmospheric pressure was measured using a GPS model Garmin E-Trex. Measurements were taken once per day in the midday hours. A Mann-Whitney *U* test was used for temperature comparison between localities.

The vocal activity was recorded using a Sharp MD portable recorder model 702 and an Olympus ME 31 shotgun microphone. The recordings were processed with the computer program Spectrogram 15.4 (Visualization Software LLC).

**RESULTS**

In March we registered consistent vocal activity in locality 1, weak diurnal and a lack of nocturnal vocalization in locality 2 and a complete lack of vocalization in locality 3 (Table 1). The water level at locality 1 was relatively shallow, only in some ponds exceeding 60 cm. There was a moderate activity of the marsh frog during the midday observations (sporadic individual vocalizing) and increased activity during the nocturnal observations (small choirs of 5-10 specimens along the canal), (Figure 1A). The calls produced were both territorial and advertisement (Figure 2B). The green toad's vocalization was only observed during the evening hours (Figure 2A). In locality 1 we registered a clear indication of habitat preferences in the distribution of the green toad and the marsh frog. The green toad vocalized exclusively from the static ponds nearer the thermal springs, while the marsh frog was distributed in greater number along the canal draining the ponds, although some specimens were recorded in the ponds as well. Some egg-clumps and tadpoles in early stages of development from both species were also observed. We did not observe any adult activity, larvae or clumps of the eastern spadefoot, however, the presence of a corpse near the ponds suggested that at least some adult specimens had been active in this locality. The water level at locality 2 was relatively deep, exceeding 1m at the centre of the pond. We registered only a weak vocal activity of the marsh frog during the midday observations – no more than four specimens were heard at any one time. The call was only territorial and we did not hear any advertisement calls at this site (Figure 1A). However, there were some clumps of the eastern spadefoot. The water level at locality 3 was also relatively deep and exceeded 1m near the centre of the pond. We did not observe any vocal activity here (Figure 1). In the shallow water near the southern bank we made a peculiar

finding – two dozens dead eastern spadefoot toads packed tightly in a 2-litre plastic bottle, all in early stages of decomposition. We also found a newly metamorphosed froglet, suggesting breeding activity of the marsh frog.

During the April observation we registered some significant changes and increased activity at localities 2 and 3, while the level of activity at locality 1 was similar to the previously observed (Table 1). Significant temperature differences were established between locality 1 and localities 2 and 3 in both March and April (Mann-Whitney *U* test: $U = 0$, $p<0.05$). It has to be noted that a part of the canal draining the springs was used for irrigation and was flooded with water from the Struma river, thereby lowering its temperature and greatly increasing its depth and current; we did not find any specimens there. At locality 2 we observed slightly increased diurnal activity of the marsh frog (sporadic individuals vocalizing), (Figure 1B). There was no nocturnal activity, although in the vicinity of the pond we heard some European tree frogs (*Hyla arborea*). The water level of the pond was significantly deeper than the previously measured, well over 1.5 m. At locality 3 we observed some diurnal activity of the marsh frog, with small choirs (5-10 specimen) vocalizing at the southern and northern side of the pond (Figure 1B). At night these were joined by consistent tree frog choirs. We also found some larvae of the agile frog (*Rana dalmatina*), but with no adult specimens seen or heard. By stark contrast with locality 2, the water level was significantly lower than the previously measured, never exceeding 50 cm.

Table 1: Measured parameters in localities 1-3 in the Rupite area. For the temperatures, outside of the brackets are the median values, and inside are min and max values. Observed activity was marked with a "+", and no activity with a "-".

| Locality | Interval of observation | Air temperature (ºC) | Water temperature (ºC) | pH | kH | Activity |
|---|---|---|---|---|---|---|
| **March** | | | | | | |
| 1 | 14-16 | 12.1 (9-14.4) | 22.06 (17.2-28.5) | 9 | 10 | + |
| | 22-24 | 9.4 (7-11) | 21.16 (20-22) | | | + |
| 2 | 14-16 | 16.05 (13.5-18.6) | 19 (18-20) | 8.5 | 5 | + |
| | 22-24 | 6.8 (6-8) | 13.2 (12-14) | | | - |
| 3 | 14-16 | 13.5 (13-14) | 15.5 (14-16) | 7.5 | 3 | - |
| | 22-24 | 6.5 (6-8) | 12 (11-14) | | | - |
| **April** | | | | | | |
| 1 | 14-16 | 17 (11-20) | 28.5 (20-37) | 9 | 10 | + |
| | 22-24 | 16.2 (10-17) | 29.5 (21.5-37.5) | | | + |
| 2 | 14-16 | 13.2 (9.5-14) | 12.8 (12-13) | 8.5 | 7 | + |
| | 22-24 | 14.5 (14-15) | 10.5 (9-11) | | | - |
| 3 | 14-16 | 14.8 (13-16) | 13.5 (12.9-14.1) | 9 | 3 | + |
| | 22-24 | 14.5 (14-15) | 11.6 (10.5-13) | | | + |

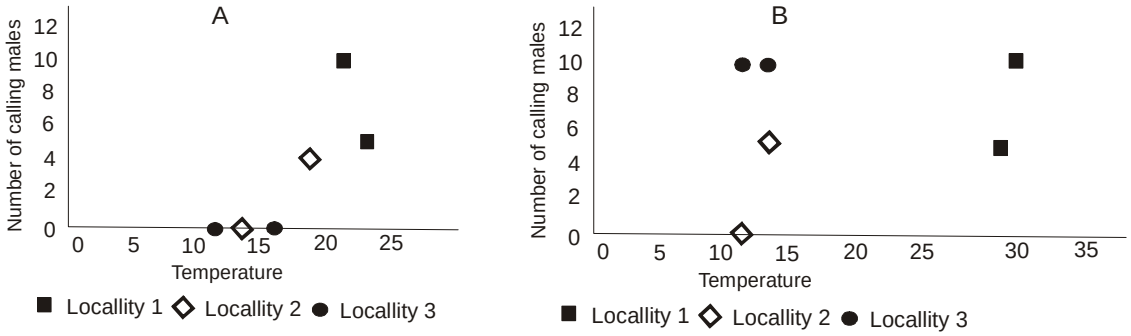


Figure 1: Relation between vocal activity of the marsh frog and temperature in the three localities in March (A) and April (B).

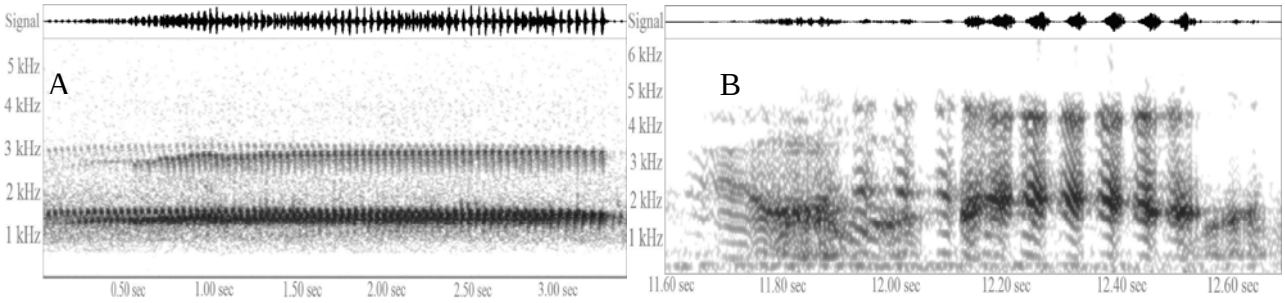


Figure 2: Sonograms presenting the frequency range and signal of the calls of the green toad (A) and the marsh frog (B). In B there is e territorial call (11.6-12s) followed by an advertisement call.

## DISCUSSION

It has been shown that the nature of habitat or surrounding land is relatively unimportant compared with the physical and chemical properties of the site [5]. In this regard it seemed locality 1 provided better conditions (high temperature, buffer qualities) than the other two, even though it was highly urbanized and with increased tourist activity, especially during the weekends. According to [12] frogs are less particular about their choice of site than toads, which would explain their presence in both the warm ponds and the canal. Since the canal provided more protection from sun and wind from plant cover, this seemed to be the preferred site for the marsh frog. Frogs that were observed in our research over the course of the day were generally found under plant cover, in cool and often moist microhabitats, and never in the direct sunlight. According to [15] at night the water acts as a temperature buffer during the nocturnal drop and that could explain the presence of frogs in the ponds as well, as water temperature decreases more slowly than air temperature. Toads generally lay their eggs in deeper water and their heavier bodies are less suited to swimming in flowing water, so we hypothesized that the ponds provided more suitable conditions for them. [5] suggested that frog tadpoles readily eat toad clumps, which could be another possible reason for the observed distribution. It has been demonstrated that calling activity is energetically costly [14] and males are at increased risk of mortality around the breeding sites. Hence, it would seem highly unlikely that males would call if there were no expectation of mating. So, even though we found no clumps neither tadpoles of both species, we have reasons to believe they were actively breeding. The presence of clumps and froglets but no activity at localities 2 and 3 during the March observation could be explained by the unusually high temperatures in the preceding weeks. The immediate environmental conditions play a significant role in the calling activity of frogs and need to be considered in conjunction with the information on calling. In this sense unfavorable climatic conditions may curtail calling activity and could prevent frogs being detected in an area where they are otherwise common, even if it is the middle of the core calling period and survey effort is high

[6]. The lack of activity at locality 2 during the April observation might be due to excessively high water levels or chemical properties of the water we could not measure. The temperatures, while low for this time of year, were similar to those at locality 3, where there was a significant increase in activity. This activity might be explained by the low water level at this locality, since frogs prefer shallower waters [10]. The scale of observation determines the patterns that can be observed. The strength of the relationship between vocal activity and microhabitat could be better understood by carrying out additional researches.

## CONCLUSIONS

The level of activity observed during both periods reflects the breeding seasons in the three species. The little difference in activity levels at locality 1 probably reflects the more stable microhabitat temperature conditions provided by the thermal springs.

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