THE FEEDING OF A *BOMBINA BOMBINA* (AMPHIBIA) POPULATION FROM IZVOARE LOCALITY, MEHEDINȚI COUNTY

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Abstract. Our study aimed to analyse the trophic spectrum of a *Bombina bombina* population from a habitat situated near Izvoare locality, Mehedinti County. The study was realised at the beginning of April. We did not register any empty stomachs. The most important animal preys are the Diptera Brahicera and Coleoptera, followed afterwards in smaller percentages by Hymenoptera, Arahnida Araneida, Diptera Nematocera, Gasteropoda. The females registered a very low percentage regarding the consumption of amphibian larvae. Beside the invertebrates, we also identified the consumption of vegetal fragments, respectively shed-skin in the analysed stomachs.

Keywords: feeding, *Bombina bombina*, Diptera Brahicera.

Rezumat. Hrănirea unei populații de *Bombina bombina* din localitatea Izvoarele, județul Mehedinți. Studiul nostru a avut ca scop analizarea spectrului trofic al unei populații de *Bombina bombina* dintr-un habitat aflat în apropierea localității Izvoare, județul Mehedinti. Studiul s-a realizat la începutul lui aprilie. Nu s-a înregistrat nici un stomac fără conținut. Dintre prăzile de natură animală cele mai importante categorii sunt dipterele brahicere respectiv coleopterele, urmate apoi în procente mai scăzute de himenoptere, arahnide araneide, dipterele nematocere, gasteropode. S-a observat consumul de către femele a unui procent foarte scăzut de larve de amfibieni. Pe lângă nevertebrate am identificat şi consumul de vegetale, respectiv exuvie în stomacurile analizate.

Cuvinte cheie: hrănire, *Bombina bombina*, Diptere Brahicere.

INTRODUCTION

The red-bellied toad, *Bombina bombina*, is one of the most spread amphibian species from Romania (COGĂLNICEANU et al., 2000). It inhabits wide field territories, but the species can also be encountered in the plateau areas, its upper altitudinal limit being of 400 m (in Transylvania) (FUHN, 1960). Due to the anthropogenic influences from the last decades, the species has become endangered in the western countries (KINNE et al., 2004). Because of the destruction of many amphibian habitats through damming and draining works, in many areas of Romania, the species is considered vulnerable (COGĂLNICEANU et al., 2000). Amphibians are sensible to the pollution of the environment, because the pollutants can be easily absorbed through the skin and gills' membrane of the larvae, and also swallowed together with the contaminated prey (BRIGGS, 2004). Due to the high sensibility, amphibians offer useful and important information regarding the habitats which they occupy, these being true indicators of the ecosystems' quality, fact that confirms that the amphibian study is very valuable.

Bombina bombina is a day-time species, living in permanent lakes and puddles of large dimensions (WILKENS, 1979; ENGEL, 1985) that are rich in amphibious vegetation; the individuals often hunt on dry land (FUHN, 1960). In Mehedinți region, studies upon the trophic spectrum of this species have not been realised so far. Therefore, the present study aims to bring new contributions regarding the feeding of the species. During the years, a series of studies have been performed concerning the feeding of the *Bombina bombina* populations (RADU et al., 2007; SAS et al., 2004; TERTYSHNIKO & GOROVAYA, 1982). In our research, we followed the differences of the trophic spectrum between the females and males, the feeding intensity regarding the sexes and the amount and frequency variation of the preys.

MATERIALS AND METHODS

Our study took place in April 2007, period that coincides with the reproduction season of the amphibians. On a whole, we captured 52 individuals, 30 males and 22 females. The individuals were captured using a net, or directly by hand in the case of the ones found near the banks. The habitat lies in the vicinity of Izvoare locality (Mehedinți County), being represented by a puddle that stretches on a surface of tens of square meters, characterised by a high level of water and an abundant aquatic and amphibious vegetation.

The collecting of the stomach contents was realised using the stomach flushing method (SOLE et al., 2005). The technique is used and recommended by many authors, because it allows the study of the amphibians' feeding without having a negative effect upon the individuals, these being released in their habitats. The stomach content was drawn using a syringe, at the edge of which a perfusion tube of different diameters was fixed, chosen depending on the frogs' size. The water was carefully and gradually injected, so as not to harm the animals, which have relatively small dimensions. We tried to reduce as much as possible the time between the capturing of the animals and the performing of the stomach contents, because the amphibians quickly digest the food (CADWELL, 1996), which can negatively influence the study. The contents were preserved in a 4% formaldehyde solution and stored in air-tight test tubes accompanied by labels with the sex of the individuals and the place of the sample collecting. The analysis of the stomach contents was realised at the binocular magnifying glass, and the scientific literature (CRIŞAN & MUREŞAN,

1999) was used for the determination of the taxonomical affiliation of the prey categories. The prey taxa were determined at the level of the class, order and where it was possible, family.

RESULTS AND DISCUSSIONS

Choosing the reproduction habitat is very important in the case of the amphibians, because the survival of the population depends on these conditions (BARANDUN & REYER, 1996). The survival of the population and especially of the larval population depends on the water quantity of the puddle, the vegetation density, the trophic resources and not last, the predators existent in the habitat. The species prefers puddles with sun exposure, preferably with dense vegetation, where the tadpoles can hide themselves (BRIGGS, 2004), and the adults can have better hunting conditions.

The prey animals identified in the analysed stomachs are mostly grouped in the invertebrate group, with a majority belonging to the Insect Class, but we have also encountered the case of the females where very low tadpole consumption was registered, 0.4 %. We identified 26 prey taxa, most of which being represented by families belonging to the Coleoptera Order, respectively Coccinelida, Curculionida, Scarabeida, Crizomelida, Cantharida, Staphilinida, Carabeida, Dytiscida, and Elaterida. The decomposition stage of some preys did not allow the identification of the families, which was able only at the Coleoptera order. In the stomach contents of the 52 individuals we identified 570 preys, 324 being consumed by the males and 246 by the females. *Bombina bombina* is a more aquatic species than her relative, *Bombina variegata* (COGĂLNICEANU et al., 2000), however, following our study, 91.4% of the preys were terrestrial, and only 8.59% were aquatic preys (Table 1). One explanation could be the fact that the habitat, from where the frogs come from, presents dense, abundant vegetation, homogenously spread on the water surface, thus the individuals had the possibility to hunt terrestrial species that were found on the grass, or the individuals hunted on the banks.

Table 1. The percentage of stomachs with vegetal fragments and animal food; The number of preys, the average and maximum number of preys/individual, the abundance of aquatic and terrestrial preys.
Tabel 1. Frecvenţa stomacurilor cu fragmente vegetale şi prăzi animale; numărul de prăzi, numărul mediu şi maxim de prăzi/individ, ponderea prăzilor acvatice şi terestre.

	Males	**Females**	**Total**
No. of individuals	30	22	52
No. of preys	324	246	570
Max. no. of preys/ individual	29	27	29
Average no. of preys / individual	11.18	10.8	10.96
% Aquatic Preys	8.33	8.94	8.59
% Terrestrial preys	91.66	91.05	91.40
% Stomachs only with vegetal matter	0	4.54	1.92
% Stomachs with animal food	100	95.45	98.07
% Vegetal	13.33	18.18	15.38
% Shed-skin	60	54.55	57.69

The feeding intensity is high; the average number of prey/individual at the males is 11.18 and at the females 10.08, the males having a predation activity slighter higher than the females. These values, together with the fact that we did not register any empty stomachs in the study, indicate that the feeding activity rate is maximum and optimum feeding and predation conditions were present. The maximum number of preys was registered at two males, at which we identified 29 preys, most being represented by Hymenoptera Formicida (22 ants). It is assumed that there could be a certain connection between the ant consumption and the skin toxicity, some papers highlighting that the alkaloids from the frogs' skin has a certain toxicity due to a feeding focused on ants (BONANSEA & VARIA, 2007).

Beside animal preys, we also identified vegetal and shed-skin fragments in the stomach contents. The frequency of the stomachs with vegetal parts is very low, and we also encountered the exclusive consumption of vegetal matter in the case of two females, while we did not identify any stomachs that contained only vegetal parts in the case of the males. In this case, the females consumed more vegetal fragments (18.18%), in comparison to 13.33%, which was recorded at the males. The consumption of vegetal parts has also been registered at other studies regarding the Bombina genus (SAS et al., 2004; PETER et al., 2006; SZEPLAKI et al., 2006). Their presence in the stomach contents of the frogs is considered by some to be the result of the accidental ingestion during the insect consumption (WHITAKER et al., 1977), or of an unsuccessful catch, when the individual captures a part of the plant on which the possible prey is found. Some authors consider that vegetal parts can also have a nutritive value (DAS, 1996) or can help to eliminate intestinal parasites (EVANS & LAMPO, 1996), thus their consumption can sometimes be voluntary.

Shed-skin is another element frequently encountered in the stomachs of the analysed frogs, the males consuming shed-skin fragments with a higher frequency (60%), while the females consumed shed-skin in a proportion of 54.55%. Numerous cases were presented in the scientific literature, in which amphibians presented shed-skin parts

beside the animal preys: *Bombina bombina, Rana arvalis* (SAS et al., 2003), *R. lessonae* (SAS et al., 2005) etc. Dermatophagy is considered to be a method of recycling the epidermal proteins (WELDON, 1993), and the increased consumption of shed-skin during the reproduction period strengthens the idea of the consumption in order to obtain a contribution of proteins necessary especially to the females for reproduction.

The most important prey category is represented by the animal organisms, adult amphibians being carnivores (COGĂLNICEANU et al., 2000). The prey taxa are mainly represented by insects, but we separated the larvae from the adults because it is considered that the larvae forms are richer in lipid, thus being more nutritive, these also representing distinct categories regarding their mobility. The prey taxa, which are found in a larvae form, belong to the Diptera Brahicera, Diptera Nematocera, Coleoptera Dytiscidae, Lepidoptera and Odonata group (Table 2). Beside invertebrates, following the analysis we also identified amphibian tadpoles, with a very low frequency of 1.92%.

Table1. The amount and frequency of the prey categories identified in the stomach contents, depending on the individuals' sex.
Tabel 1. Ponderea şi frecvenţa taxonilor pradă identificaţi în conţinuturile stomacale, în funcţie de sexul indivizilor.

Prey categories	Amount (%)			Frequency (%)		
	Males	Females	Total	Males	Females	Total
Oligocheta-Lumbricida(t)	0.93	2.84	1.75	6.66	18.18	11.15
Gasteropoda (aq)	1.85	2.43	2.11	16.67	9.09	13.46
Gasteropoda (t)	4.32	13.41	8.25	26.67	54.55	38.46
Arahnida-Araneida (t)	3.34	8.53	5.61	30	59.09	42.31
Arahnida-Acarian (t)	1.23	3.65	2.28	13.33	31.82	21.15
Crustacean-Ostracoda (aq)	2.16	0.81	1.58	3.33	4.54	3.94
Odonata (L) (aq)	0.31	0	0.18	0.18	0	1.92
Homoptera-Cicadina (t)	0.62	0.4	0.53	6.66	4.54	5.76
Heteroptera (aq)	1.54	2.84	2.11	13.33	22.73	17.31
Coleoptera-Carabeida (t)	0	2.03	0.88	0	18.18	7.69
Coleoptera-Dytiscida (L) (aq)	1.54	1.22	1.4	16.67	13.64	15.38
Coleoptera-Staphilinida (t)	0	0.4	0.18	0	4.54	1.92
Coleoptera-Scarabeida (t)	3.4	1.22	2.46	10	9.09	9.61
Coleoptera-Coccinelida (t)	0.31	0.4	0.35	3.33	4.54	3.84
Coleoptera-Curculionida (t)	0.93	1.22	1.05	6.66	13.64	9.61
Coleoptera-Crizomelida (t)	0.62	0.4	0.53	6.66	4.54	5.76
Coleoptera-Cantharida (t)	0	0.81	0.35	0	9.09	3.84
Coleoptera-Elaterida (t)	0.31	0	0.18	3.33	0	1.92
Coleoptera	3.4	4.87	4.04	23.33	36.36	28.85
Diptera-Nematocera (t)	5.56	2.03	4.04	36.67	13.64	26.92
Diptera- Nematocera (L) (aq)	0.93	1.22	1.05	6.66	13.64	9.61
Diptera-Brahicera (t)	27.8	22.76	25.6	70	68.18	69.23
Diptera-Brahicera (L) (t)	7.41	0	4.21	10	0	5.76
Hymenoptera (t)	5.25	7.31	6.14	40	54.55	46.15
Hymenoptera – Formicida (t)	25.6	16.67	21.8	43.33	27.27	36.54
Lepidoptera (L)	0.62	0	0.35	0.35	0	3.84
Trichoptera(t)	0	2.03	0.88	0	18.18	7.69
Amphibians (L)	0	0.4	0.18	0	4.54	1.92

Their consumption could be explained through the fact that they represent an easily prey to swallow, but if we relate to the low percentage, the consumption could be from necessity, in the lack of other preys.

The most important value of the amount is registered by the adult Diptera Brahicera, 25.6%, the males consuming these preys in a somehow larger percentage (27.8%) than the females (22.76%). The flies are closely followed by the Hymenoptera Formicida, which register an amount of 21.8%, and also in this case the males consumed these preys in a larger number, 25.6%, in comparison to the females that registered a consumption of 16.67%. On the other hand, the females preferred the consumption of Gasteropoda (13.41%), which registered higher percentages than the males, 4.32%, respectively the Arahnida Araneida recorded a value of 8.53% in comparison to 3.34% in the case of the males, these being larger preys and easier to capture. It is obvious that the males consumed smaller preys, that is

why in order to satisfy the energetic needs a more active consumption was necessary and a higher number of these prey taxa. The females preferred larger preys, thus saving the very important energetic resources during the reproduction period. In the stomach contents of the males we encountered Brahicera in the larvae stage with a valuable amount of 7.41%, these being important because of their lipid content, while the females did not consume these larvae at all. The males can thus complete their trophic necessities through the larvae consumption, balancing the consumption of small preys and relatively poorer in nutritive substances. The shed-skin also contributes to accomplishing this equilibrium, which is higher in the case of the males. We can observe the high values of the Brahicera at both sexes. It is considered that the Diptera, respectively Nematocera occupy a very important place in the trophic spectrum of the amphibians (LOW et al., 1990), the performed study confirming this aspect.

The taxa belonging to the Hymenoptera also registered important amount values, with a percentage of 6.14%, these being mostly consumed by the females, followed by the Nematocera, Araneida and Coleoptera. In the case of the Coleopterans we managed to identify several families, these representing on a whole 37.71% from the food of the studied population. Important values of the Coleopterans were also registered at other *Bombina* populations (GONCHARENKO et al., 1978; SZEPLAKI et al., 2006). The beetles are preys easy to capture and are abundantly found, being the order with the most numerous species from the entire living world (RADU & RADU, 1967). From the representatives of the Coleoptera, several families that have species that are harmful to the agricultural cultures are present (Elaterida, Crisomelida, Curculionida) (SAS et al., 2003), thus underlining the importance of the red-bellied frog. Other categories identified in the stomach contents, but with a low amount are the Trichoptera, Homoptera-Cicadina, Crustacean-Ostracoda, Lepidoptera, Odonata, Arahnida-Araneida, Oligocheta-Lumbricida, these being consumed by both sexes in reduced percentages. It can also be observed the consumption of both aquatic and terrestrial species; therefore we can state that the individuals did not prefer to choose their food, taking advantage of any opportunity to capture the prey.

Regarding the frequency, the Diptera Brahicera are the most frequently encountered in our study, 69.23%, followed by the Hymenoptera 46.15% respectively Hymenoptera-Formicida with 36.54%. The Arahnida-Araneida, 42.31%, and the terrestrial Gasteropoda, 38.46%, also present high frequencies. Both terrestrial snails and spiders registered higher frequency and amount values in the case of the females' feeding, these being easily captured preys, the females having a tendency not to waste the energetic reserves in the hunting process, especially using the "sit and wait" hunting technique, while the males hunt more actively through the "active foraging" strategy (PERRY & PIANKA, 1997). The males frequently consumed aquatic Gateropoda, registering a value of 16.67% in comparison to the value recorded by the females, 9.09%, the Coleoptera Dytiscida in the same percentage, aquatic insect larvae. In the case of the males, the aquatic invertebrates are more frequently consumed in comparison to the females, which prefer a more terrestrial prey. Another well represented group in the food of the males is represented by the Nematocera, 36.67%, in comparison to the females, at which the frequency of these preys is much reduced. The females also frequently consumed Araneida 59.09%, Acarian 31.82%, Lumbricida, and Trichoptere with the same frequency value 18.18%.

CONCLUSIONS

Following the obtained results, we can conclude that between the two sexes there are not very high differences regarding the prey origin or the feeding intensity, the recorded values being very similar. Both sexes mostly consumed terrestrial preys, existing differences concerning only the capturing technique of the preys. Thus, the males hunted using the "active foraging" strategy, while the females tried to maintain their energetic resources necessary for the reproduction using the "sit and wait" technique. These results prove once more the opportunistic character of the amphibians. The males generally consumed smaller preys, while the females larger ones.

The most well represented prey taxa, regarding both the amount and frequency, are the Diptera Brahicera, together with the Coleoptera, these being represented by different families. The Hymenoptera are also well represented, especially the Formicida, followed by the Arahnida Araneida, where we can notice slight differences between the two sexes. We also identified beetle species that are harmful to the agricultural crops. Beside animal preys, we also found in the stomach contents vegetal and shed-skin fragments. The vegetal parts are mostly consumed by the females, while the males consumed more shed-skin.

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