FOOD ANALYSIS IN ADULTS (MALES/FEMALES) AND JUVENILES OF BOMBINA VARIEGATA

BY

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The objective of our study was to bring data upon the feeding of *Bombina variegata* population from the Beius Depression region. We observed the trophic spectrum variations depending on the sex and the size of the toads. In the analysed stomachal contains we've found both animal preys, vegetal fragments and shad skins. We found the highest diversity of prey taxons in adults, especially yellow belied toad females. The males of the analysed toads mostly use the so called "active foraging" feeding method. The females and the juveniles apply the "active foraging" method but the "sit and wait" technique as well. The studied toads consumed mostly terrestrial animal preys.

Introduction

The key factor of the ecology of Amphibians is feeding, they spending a lot of time of their life securing it (Perry et al. 1990). Most Amphibians resort to a big diversity of trophic resources because they feed on in both aquatic and terrestrial biotopes. The cause of amphibian sexual dimorphism may be the use of different resources of individuals from populations of different sexes (Shine 1989). This is how some differences may appear between the sexes when talking about feeding (different prey types, different feeding ratio). On the other hand, among amphibian populations, individuals of different sizes consume different prey categories (Fraser 1976). It can be said that there are variations in the composition of the food all along their ontogenesis (Lőw & Török 1998).

Bombina variegata is one of the most common Amphibian species from the herpetofauna of Romania, being widely spread in the hills and the mountain regions (Cogălniceanu et al. 2000a). Thus, there are few studies about the trophic spectrum of this species in Romania (Sârbu 1976, Sas et al. 2004a, Nemes & Petráss 2003) just as well as little information about *Bombina variegata*'s food world-wide (Taraščuk 1959, Kminiak 1978, Ščerbak & Ščerban 1980, Kuzmin 1990).

The goal of our study was to bring new data on the trophic spectrum of

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Bombina variegata, comparing the used trophic resources and the intensity of the feeding between the sexes and taking into consideration the specimens sizes.

Material and methods

The specimens of *Bombina variegata* investigated by us come from the surroundings of Sustius (Beiuş Depression, Bihor County, Romania). Populations of the yellow belied toad were recently identified in this region by Covaciu-Marcov and co-workers (2003).

The study took place in the warm period (April - October) of 2004, analysing the trophic spectrum in 285 specimens of *Bombina variegata* (141-males, 106-females, 39-juveniles).

The stomachal contains were collected using the stomachal flushing method (Legler & Sullivan 1979, Opatrny, 1980). The advantage of this method is the fact that it makes possible the study of feeding in amphibians without having to kill the animals (Cogălniceanu et al. 2000b). Once analysed, the individuals are set free, back in the habitat were they came from.

We used syringes of 20-50 cm3 with a perfusion tube of different lengths and exterior diameters (40-60mm X 1.5-2.5mm) attached on them, depending on the size of the analysed toads. We tried to minimize the time between the capture and the stomach al wash because the frogs can digest their prey in a relatively short period, this being able to interfere with the obtained results (Caldwell 1996). The collected stomachal contains were conserved in formalin 4 % and stocked in sealed test-tubes with labels with information about the specimen's length and sex. The young *Bombina variegata* specimens whose sex wasn't possible to identify there were marked as juveniles.

The analysis of the stomachal contains was done in laboratory conditions, using a stereomicroscope and with the help of specific literature (Móczár et al. 1950, Radu & Radu, 1967). The analysed toads were separated in only two groups: adults (males and females) and juveniles, because of the small differences in length between the adults and the juveniles. To analyse the diversity of the prey we calculated the Shannon & Weaver (1949) index: $H = -\Sigma(n_i/N) \ln(n_i/N)$, where n_i is the number of specimens from an *i* prey category and N is the total number of preys.

Results

All the analysed toads had stomachal contains. In these stomachal contains we found vegetal fragments, remains of shad skin (Tab. 1) and animal native preys (Tab. 2).

Table 1. Weight of the	stomachal	contains	with	vegetal	fragments	and shad	skins
(SD-standard deviation)							

		Sex		Size	
	Males Fema	ales SD	Adults	Juveniles	SD
% stom. with plants	60.28 54.	71 3.93	58.13	38.46	13.9
% stom. with shed-skin	32.62 19.	81 9.05	27.23	20.51	4.75

The biggest weight of the stomachs with vegetal remains belonged to the adults of the analysed toads. This weight presents a big standard deviation then it comes to the juveniles of the yellow belied toads. The difference between the weight of the vegetal fragments from the males and the females is not significant. The males of *Bombina variegata* consume much more frequently the exuviae of other indivuals from the population than the females or the juveniles.

The most important category of stomachal contains was represented by food of animal origin. The consumed prey animals, grouped in 47 categories, there were represented by different groups of invertebrates (Tab. 2). Exceptionally, we found two males that had consumed a tadpole each. The adults (mostly females) presented a grater diversity of consumed prey animals.

· · ·		Sex			Size	
-	Males	Females	SD	Adults	Juveniles	SD
Lumbricida	4.98	4.55	0.3	4.77	0.98	2.68
Gasteropoda	15.64	9.47	4.35	12.64	1.63	7.78
Izopoda	2.06	0.91	0.81	1.5	-	1.06
Amphipoda	1.03	1.09	0.04	1.06	-	0.75
Ostracoda	-	-	-	-	4.26	3.01
Copepoda	0.1	-	0.12	0.08	-	0.06
Cladocera	-	-	-	-	0.98	0.69
Acaria	-	0.36	0.25	0.17	1.63	1.03
Araneida	5.15	6.19	0.73	5.65	1.63	2.84
Opilionida	-	0.18	0.12	0.08	-	0.06
Miriapoda Chilopoda	-	0.91	0.64	0.44	-	0.31
Miriapoda Diplopoda	0.17	0.18	0.007	0.17	-	0.12
Colembola	6.7	8.56	1.31	7.6	24.59	12.01
<i>Efemeroptera</i> -larvae	5.49	1.27	2.98	3.44	-	2.43
<i>Efemeroptera</i> -imago	0.51	1.09	0.4	0.79	-	0.56
Ortoptera-larvae	0.17	-	0.12	0.08	-	0.06
Ortoptera-imago	0.34	0.72	0.27	0.53	0.32	0.14
Coleoptera-larva undet.	1.71	1.45	0.18	1.59	0.65	0.66
<i>Coleoptera Dytiscida-</i> larvae	1.37	0.91	0.32	1.14	-	0.81
Coleoptera-imago undet.	14.78	11.83	2.07	13.35	13.44	0.06
Coleoptera Dytiscida	-	0.18	0.12	0.08	-	0.06
Coleoptera Stafilinida	0.68	0.18	0.35	0.44	0.32	0.08
Coleoptera Cantarida	-	-	-	-	0.32	0.23
Coleoptera Elaterida	0.34	1.27	0.65	0.79	-	0.56
Coleoptera Scarabeida	-	0.36	0.25	0.17	-	0.12
Coleoptera Carabidae	0.51	0.91	0.27	0.7	-	0.5
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Table 2. The amount of the prey taxa encountered in the stomach contents	(SD-
standard deviation)	

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		Sex			Size		
	Males	Females	SD	Adults	Juveniles	SD	
Coleoptera	0.34	0.18	0.11	0.26	_	0.18	
Coccinellidae	0.54	0.10	0.11	0.20		0.10	
Coleoptera	0.17	0.18	0.007	0.17		0.12	
Curculionidae	0.17	0.10	0.007	0.17	-	0.12	
Coleoptera		0.18	0.12	0.08		0.06	
Crysomelidae	-	0.10	0.12	0.08	-	0.00	
Dermaptera	0.51	-	0.36	0.26	0.32	0.04	
Heteroptera	1.54	1.27	0.19	1.41	0.65	0.53	
Homoptera Afida	1.2	2.18	0.69	1.67	4.26	1.82	
Homoptera Cicadina	1.37	1.45	0.05	1.41	2.29	0.62	
Lepidotera-larvae	0.34	0.36	0.01	0.35	0.98	0.44	
Lepidoptera-imago	0.17	0.18	0.007	0.17	0.32	0.1	
Trichoptera	0.17	-	0.12	0.08	-	0.06	
Diptera Nematocera-	3.78	2.91	0.61	3.35	2.95	0.28	
larvae	5.70	2.91	0.01	5.55	2.93	0.28	
Diptera Brahicera-	2.20	5 20	1.40	4.24	2 27	0.00	
larvae	3.26	5.28	1.42	4.24	3.27	0.68	
Diptera Nematocera-	C 01	16.02	7 70	11.21	751	2 (7	
imago	6.01	16.93	7.72	11.31	7.54	2.67	
Diptera Brahicera-	2 26	2.00	0.11	2 10	12 77	7 40	
imago	3.26	3.09	0.11	3.18	13.77	7.48	
Hymenoptera-undet.	0.51	2	1.05	1.23	1.63	0.28	
Hymenoptera	14 42	Q 10	4.4	11.4	10.91	0.41	
Formicidae	14.43	8.19	4.4	11.4	10.81	0.41	
Hymenoptera	0.17		0.12	0.09		0.07	
Ihneumonida	0.17	-	0.12	0.08	-	0.06	
Hymenoptera Apida	-	0.18	0.12	0.08	-	0.06	
Plecoptera-larvae	-	2.36	1.67	1.14	0.32	0.58	
Plecoptera-imago	0.51	0.36	0.1	0.44	-	0.31	
Tadpoles	0.34	-	0.24	0.17	-	0.12	

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To estimate the intensity of the feeding of the analysed specimens of *Bombina variegata*, we calculated the maximum and the average number of preys that they consumed (Tab. 3). The average number of preys / individual is highest when it comes to the juveniles and the females of the yellow belied toads. We encountered the same situation in the case of the maximum number of preys / individual.

 Table 3. Maximum and the average number of preys / individual (SD-standard deviation)

	Sex			Size			
	Males	Females	SD	Adults	Juveniles	SD	
Max. no. of preys / individuals	4.05	5.16	0.78	4.59	7.82	2.27	
Average of preys / individuals	17	24	4.95	24	20	2.82	

The toads investigated by us preferred to eat preys with reduced mobility. Only the females and the juveniles consumed a significant number of preys with bigger mobility. The juveniles of the yellow belied toad consumed a much grater number of prey animals with gregarious life stile than their adults (Tab. 4, Fig. 2).

Table 4. The standard deviation (SD) of the amount of preys depending their mobility and life stile

		Mobility		single	aquatic/ terrestrial
	low	medium	high	/gregarious	
Males / Females	8.68	0.26	8.94	0.58	2.78
Adults / Juveniles	0.68	5.05	4.37	15.36	3.49

Among the most important prey invertebrates consumed by the investigated toads were: Lumbricida, Gastropods, Araneida, Collembolas, Efemeropteras larvae, Coleopteras, Hymenopteras and the imago forms of Diptera Nematoceras and Brahiceras. In the weight of these preys there are differences depending on the sex and the sizes of the analysed toads. Thus, the consumption of Lumbricidas, Gastropods and Araneidas is important only for the adults of *Bombina variegata*. Their juveniles presented a significant preference to Collembolans.

The analysed toads have hunted on the ground and in the water as well (Fig. 2). Animals of terrestrial origin appear with higher weight.

Discussions

Generally speaking, the food of Amphibians is regular, composed of different groups of invertebrates. The presence of vegetal fragments in the stomachal contains of the analysed toads is simply accidental. These fragments were swallowed simultaneously with the animals (Whitaker et al. 1977), Amphibians are feeding on mobile preys (Zimka 1966). The lack of stomachs with only vegetal contains supports this statement. We encountered similar cases in other populations of *Bombina variegata*, too (Sas et al. 2004a).

Some consider that the consumption of exuvial fragments is caused by the recirculation of epidermal proteins (Weldon et al. 1993). With all that, we consider that dermatofagy is only accidental, the toads acting on the movement of other individuals of their population. They, of course, can not capture them so they end up swallowing skin

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fragments stuck to their tongue. This statement is also supported by the fact that at the majority of the adult toads analysed by us we found the proof of dermatofagy. Other cases of dermatofagy were discovered is other populations of *Bombina variegata*, as well (Sas et al. 2004 a).

Bombina variegata presents a large diversity of preys (Tab. 5), being a general predator.

 Table 5. Diversity of feeding depending on sex and size (SD-standard deviation)

	Sex			Size			
	Males	Females	SD	Adults	Juveniles	SD	
Feeding diversity (H)	3.64	3.79	0.1	3.77	3.42	0.25	

The studied toads (mostly males) use the "active-foraging" hunting technique, eating preys with low mobility, with or without grouped distribution (Huey & Pianka 1981). Females and juveniles of *Bombina variegata* use mostly the "sit-and-wait" hunting method, consuming a lot of animals with high mobility, which they grab the moment they see them (Perry & Pianka 1997). Using the "sit-and-wait" technique reduces the loss of energy used for capturing food. This is important for females (a lot of energy is used for laying eggs) and juveniles, too (they use most of the energy for growing).

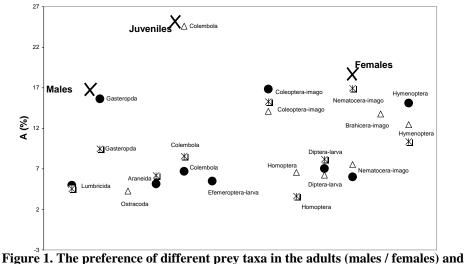
The optimum foraging theory (Pyke 1984) assumes that the maximum efficiency of energy consumption is encouraged by the natural selection. Thus, we can accept the fact that the size of the preys plays an important role in the feeding strategy of the yellow belied toads.

The *Bombina variegata* adults present a bigger diversity when it comes to food. The adult specimens consume larger preys (worms, bugs, snails, butterflies), while the juveniles consume mostly smaller animals, with gregarious life (Collembolans). However, we can't talk about a certain preference towards some prey taxons depending on the sex or size. The juveniles are restricted to smaller preys due to their small mouth, eating larger preys only occasionally. Once with the growth of the predator's size, the prey taxons interval enlarges. Smaller preys can be captured by both larger and smaller toads (Lőw et al. 1990). The toads, as they age and grow, eat fewer smaller preys and more bigger prey animals. Thus, there is a direct link between the size of the prey taxons and the one of the predator. Therefore, it can be stated that there are variations along *Bombina variegata*'s ontogenesis in the composition of their food. The energy loss is smaller when it comes to hunting fewer larger preys than when hunting many smaller animals (Lőw & Török 1998).

Similar situations, when females, but also Amphibian adults in general, present a bigger diversity of the consumed prey taxons and the use of different hunting techniques were observed in other species of Amphibians (Lőw et al. 1990, Lőw & Török 1998, Cicort et al. 2004).

The taxonomic composition of the preys is in direct correlation with their accessibility (their size and their abundance in the habitat), a fact that represents a natural

selection at the yellow belied toad's trophic spectrum level. The Amphibians capture all animals from their biotope that have a suitable size for swallowing (Török & Csörgő 1992). Therefore it is possible for them to consume not only invertebrates but some small vertebrates as well. Thus, we found cases of Amphibian tadpoles in the stomachal contains investigated by us. Comparable cases were described in other species of Amphibians, too (Covaciu – Marcov et al. 2002, Sas et al. in press).



juveniles of *Bombina bombina* (circles – males, asterisks – females, triangles juveniles)

As *Bombina variegata* is an aquatic species (Cogălniceanu et al. 2000a), it is more likely for it to consume mostly aquatic preys. However, terrestrial prey animals have quite a big weight in this Amphibian's menu. This doesn't necessarily mean that the toads hunted on the ground. A lot of animals of terrestrial origin are accessible to the toads at the luster of the water (spiders) or from on the aquatic plants (flying insects). Other Amphibians related to the water consume mostly terrestrial preys, too (Lőw et al. 1990, Sas et al. 2004c).

After studying the trophic spectrum of both vicariant species of the *Bombina* genus, we established that the populations of *Bombina variegata* consume generally terrestrial preys (this study, Sas et al. 2003a) while the populations of *Bombina bombina* prefer aquatic preys (Sas et al. 2003, 2004b). This fact is in positive correlation with the accessibility of both aquatic and terrestrial preys from the habitats covered by the two species.



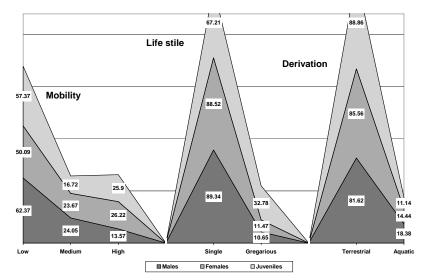


Figure 2. The amount of preys in function of their mobility, life stile and derivation

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