

The food composition of some *Bombina* populations from Livada forest (Satu Mare county, Romania)

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Abstract. We analysed the trophic spectrum of six *Bombina* hybrid populations from Livada Forest. The most frequently consumed preys are dependent of the habitat, the omnipresent ones were Coleopterans, Dipterans and Arachnids. The Collembolans accidentally register a high abundance. The shed skin consumption is indirectly connected with the feeding intensity, suggesting the fact that it can be an additional food aspect, being rich in protein. Regarding the differences between the sexes, the large-sized preys present a majority in the case of the females and juveniles, respectively those rich in lipids (Lepidoptera larvae). Thus, their capture requires a lower amount of energy, which is needed in the growth and reproduction process. Most of the preys have a terrestrial origin.

Key words: *Bombina*, hybrids, trophic spectrum, opportunistic feeding.

Introduction

The fire-bellied toads *Bombina bombina* and *Bombina variegata*, interbreed in a long, narrow zone maintained by a balance between selection and dispersal (Hofman et al. 2007). The hybrid zone is established partly by endogenous selection against hybrids (Kruuk et al. 1999) and, presumably, also by selection against toads in the wrong habitat: *B. bombina* typically reproduce in semi-permanent ponds in the lowlands, whereas *B. variegata* lay their eggs in ephemeral water bodies at higher elevations (Nürnberg et al. 2005). In the Livada forest, there were identified both populations close to *B. bombina* or *B. variegata*, as well as hybrid populations, once with the altitudinal increase there is also an increase of the *B. variegata* features (Covaciu-Marcov et al. 2008). The morphology of the habitats have a decisive role in the selection of the hybrid phenotypes and therefore in the aspect of the populations (Covaciu-Marcov et al. 2008).

Studies regarding the *Bombina* hybrids have been made before in our country (Ghira & Mara 2000, Covaciu-Marcov et al. 2002a, 2004, 2005, 2008), but data regarding the trophic spectrum of the *Bombina* hybrids are very few (Sas et al. 2005a, Ferentzi et al. 2007). The research especially refers to the trophic spectrum of the parental species *Bombina bombina* (Sas et al. 2004a, Szeplaki et al. 2006, Radu et al. 2007, Tertysnikov & Goroyava 1982, Kovács & Török 1992, Goncharenko et al. 1978, Orsagova 1969, Lác 1958, Medvedev 1974,

Ratajsky & Vojtkova 1971) as well as *Bombina variegata* (Sas et al. 2004b, 2005b, 2006, Peter et al. 2005, 2006, 2007, Tóth et al. 2007, Groza et al. 2006)

Our objective is to analyse the differences that appear in the trophic spectrum of some hybrid *Bombina* populations in a relatively limited area, and respectively the influence of local variations on the feeding behaviour of these frogs. The results were analysed from several points of view, such as the differences between the sexes, the stage of ontogenetic development or the origin of the habitat.

Materials and methods

Our study took place during two periods, in 27 - 28 April respectively 11 - 12 May 2007. We collected 247 *Bombina* individuals, from 6 different habitats, from the Livada forest, Satu Mare County.

Habitat 1 is a temporary puddle situated near a forestry road, between the forest and the railroad. The vegetation is very poor, the biotope having a very high degree of pollution, sometimes cars which disturb the water pass by. 30 individuals were taken from this habitat.

Habitat 2 is represented by a marsh situated inside the woods, having a surface of ten times the size of the previous one, approximately 30 m², with a very rich vegetation and fauna. Because this habitat is more isolated from the road, it has a very low level of pollution. From this habitat 23 individuals were analysed.

Habitat 3 is actually a system of temporary puddles situated alongside the forestry road, having a very small

surface and poor vegetation. These puddles are in connection with a larger one, which presents vegetation to a certain extent (pewter, reed). The water is shaken by the cars which drive through it. 34 *Bombina* individuals were captured from this habitat.

Habitat 4 is a canal situated alongside the road, lacking vegetation. The substratum is stony, and the level of the water is variable depending on the precipitation quantity. The *Bombina* population is the highest, registering 61 captured individuals.

Habitat 5 is represented by a puddle situated in an abandoned quarry, and thus lacking vegetation, with disturbed water. The number of analysed individuals is 40.

Habitat 6 is also a system of puddles situated inside an abandoned quarry, having the largest size and being very similar to the previous one concerning the conditions. From here we collected 59 *Bombina* individuals.

We used the stomach flushing method recommended by many authors from the scientific literature (Mahan & Johnson 2007, Solé et al. 2005, Solé & Pelz 2007, Caputo & Vogt, 2008). The drawn samples were conserved in a 4% formaldehyde solution. The determination of the preys was made in the laboratory using the scientific literature (Radu & Radu 1967, Steinbach et al. 2000, Chinery, 1998; Paulian, 1971).

The results were statistically processed and compared depending on the origin of the habitat, the sexes and respectively the stage of ontogenetic development. We analysed several parameters: the taxonomic affiliation of the consumed preys, the feeding activity rate, the intensity of the feeding, the abundance, the origin and the prey frequency, the food diversity (Shannon Weaver index).

Results

The food composition of the hybrid *Bombina* populations presents many variations depending on the origin of the habitat, the sexes and the stage of ontogenetic development. Concerning the taxonomic affiliation of the preys, we classified them in several taxonomic groups, their number depending on the habitat. This classification was made only until the order level, in some cases even families.

In the stomach samples, we identified the presence of vegetal remains, shed-skin and minerals (Tab. 1). The consumption of vegetal remains is higher in the case of the larger habitats. On the other hand, we can observe a higher consumption of vegetal fragments in the case of the females and juveniles where the habitat is larger (habitat 1, 2, 5 and 6).

Regarding the shed skin consumption the values are higher in the case of the adults. Also, there can be observed a negative connection between the feeding intensity and the consumption of this element (habitat 2 and 3). Concerning the sexes, the stomach frequencies with shed skin varies, being higher in the case of the females from the 4th, 5th and 6th habitat. And regarding the 1st, 2nd and 3rd habitat the frequency is higher in the case of the males. The minerals appear with a very low frequency only in the case of habitat 6.

Table 1. The percentage proportion of the aquatic and the terrestrial prey items. The feeding intensity of the consumed preys. The frequency of vegetal remains and shed-skin [M – males, F – females, J – juveniles].

	Habitat 1			Habitat 2			Habitat 3		
	M	F	J	M	F	J	M	F	J
% Terrestrial preys	67.20	58.20	62.20	99.20	99.80	94.90	74.50	90.70	83.30
% Aquatic preys	32.80	41.80	37.80	0.82	0.21	5.13	25.50	9.30	16.70
The maximum no. of preys	19	17	12	253	367	11	7	7	3
The average no. of preys	12	9.2	6.4	38	85	5.6	3.9	4.8	3
% Vegetal fragments	54.54	66.66	85.71	37.50	63.63	57.14	75.00	55.55	50.00
% Shed-skin	18.18	16.66	-	18.75	9.09	14.28	41.66	22.22	-

	Habitat 4			Habitat 5			Habitat 6		
	M	F	J	M	F	J	M	F	J
% Terrestrial preys	95.80	93.90	97.60	99.50	97.80	93.80	100.00	96.80	99.40
% Aquatic preys	4.17	6.09	2.35	0.51	2.18	6.25	-	3.16	0.56
The maximum no. of preys	29	32	12	26	30	16	16	24	26
The average no. of preys	12	11	6.1	12	13	9.6	8.6	8.3	9.3
% Vegetal fragments	44.44	31.03	42.85	35.29	44.44	20.00	28.57	78.95	42.11
% Shed-skin	27.77	31.03	14.28	17.64	22.22	20.00	23.80	47.37	15.79

With concern to the origin of the preys, they occur both from the terrestrial and aquatic environment. Generally, the preys are mainly terrestrial, but in the case of habitat 1 we can observe a relatively high abundance of the aquatic preys.

With regards to the feeding intensity we took into consideration two parameters, thus the maximum and average number of preys/individual. In the case of the second habitat the feeding intensity is very high due to the high number of Collembolans, which are small-sized preys and therefore are consumed in a very large number. The size of the preys also influences the feeding intensity in the case of the 4th, 5th and 6th habitat, but here the Formicidas appear. Concerning the average number of preys, we can observe a higher value in the case of each habitat at the females, respectively, this value is higher in the case of the adults than in that of the juveniles.

In the analysis of the animal preys we calculated their abundance, respectively the frequencies with which they appear in the stomach contents (Tab. 2). The preys with high abundances vary depending on the habitat and sex. The terrestrial Coleopterans represent preys with a relatively high abundance. The Nematocera larvae register the maximum abundance in the case of habitat 1. The Collembolans register the maximum abundance in the second habitat, their very high number determines the abundance of other preys to be more reduced (including that of the Coleopterans). The Brahicerans adults have high abundances in the 3rd, 4th and 5th habitat, the highest being in the 5th one. In the 6th habitat, after the Coleopterans, the Afida Homoptera appear in the second place. The appearance of the Formicidas in the 1st, 4th and 6th habitats is very interesting. Regarding the differences between the sexes, the larvae of some insects (Lepidoptera, Diptera Nematocera) register higher abundances in the case of the females.

The frequencies of the prey taxa are different in several cases regarding the value of the abundance. In the case of each habitat the value of the frequencies is relatively high at the Coleopterans, these being omnipresent in each habitat. The very high frequency of the Brahicerans in the case of the second habitat is interesting, in the second place the Coleopterans can be found, and the Collembolans do not present a very high frequency, even if they have a maximum abundance. On the other hand, the Brahicerans have a much higher frequency than the value of their abundance.

Not all of the individuals presented stomach contents. The frequencies of empty stomachs varies regarding the habitat, the highest value being present in the case of the 3rd, 6th and 5th habitat, but there are also situations in which all of the individuals presented

stomach contents (habitat 1).

In order to estimate the diversity of the consumed preys, we calculated the Shannon Weaver index (H) (Tab. 3). The low value in the case of the 2nd habitat is very interesting, and the relatively high value in the case of the 3rd habitat, even if in this habitat the feeding intensity is lower.

Discussions

The ingestion of vegetals cannot be regarded as active due to the consideration that the frogs are carnivores (Cogălniceanu et al. 2000) that feed only on mobile preys (Zimka 1966). The vegetal remains, which do not have a trophic role (Anderson et al, 1999), are accidentally swallowed together with the followed mobile prey (Whitaker et al. 1977, Stebbins & Cohen 1995). Regarding the sexes, we can observe a higher frequency of the vegetals in the case of the females and the juveniles, but only in the larger habitats. In these habitats the vegetation is richer, thus the possibility of swallowing vegetal fragments increases. On the other hand, potential preys are present on the surface of the vegetals, which can represent an important food source for the females and juveniles, these having higher energetic needs for reproduction and growth. In the case of the males the presence of this element within the trophic spectrum is more homogenous, this suggesting that their feeding is more selective. This can result from the different hunting strategy (active foraging). The appearance of the vegetals in the stomach contents has been noticed in many feeding studies (Covaciu-Marcov et al. 2002b, Széplaki et al. 2006, Peter et al. 2006).

The active consumption of shed-skin fragments has been signalled at several amphibian species, especially in unfavourable conditions regarding the feeding of the Urodela (Gunzberger 1999, Cicort-Lucaciu et al. 2006 a, b), but also in that of the Anura (Sas et al. 2003). The last three habitats have a high degree of pollution that can also influence the trophic offer. The females, which need energy for reproduction, also consumed shed-skin as a way of recycling the epidermal proteins (Weldon et al. 1993). It is interesting that in the 2nd and 3rd habitats, the shed skin consumption is indirectly proportioned with the feeding intensity. The specific characters of each biotope can be a probable explanation. Thus, the smaller population and larger puddle from habitat 2 has lower possibilities of swallowing shed skin than the one from habitat 3, which is larger and lives in smaller puddles, and therefore the chances of ingesting this element increase. The size of the consumed preys determine the differences in the feeding intensity. These are larger in the second habitat where Collembolans were consumed,

Table 2. The percentage abundance (A%) and the frequency of occurrence (F%) of the prey items.
[M- males, F- females, J- juveniles, L.-larvae, aq- aquatic, t- terrestrial]

	Habitat 1		Habitat 2		Habitat 3	
	A%	F%	A%	F%	A%	F%
Anelida	0.35	3.33	-	-	-	-
Bivalva	-	-	-	-	2.08	8.69
Gasteropoda [t.]	-	-	-	-	-	-
Gasteropoda [aq.]	0.35	3.33	-	-	1.04	4.34
Izopoda [t.]	0.35	3.33	0.25	5.88	2.08	8.69
Izopoda [aq.]	-	-	0.31	8.82	3.12	4.34
Arachnida - Acaria	-	-	0.06	2.94	-	-
Arachnida - Araneida	0.70	6.66	0.50	20.59	-	-
Arachnida - Opilionida	-	-	-	-	-	-
Arachnida - Pseudoscorpionida	-	-	-	-	-	-
Miriapoda - Chilopoda	-	-	-	-	-	-
Miriapoda - Diplopoda	-	-	-	-	-	-
Collembola	-	-	89.14	38.24	2.08	4.34
Ephemeroptera [L.]	-	-	-	-	2.08	4.34
Plecoptera	-	-	-	-	-	-
Plecoptera [L.]	-	-	-	-	-	-
Odonata	-	-	-	-	-	-
Odonata [L.]	0.70	3.33	-	-	1.04	4.34
Ortoptera	-	-	-	-	-	-
Heteroptera [t.]	0.35	3.33	-	-	1.04	4.34
Heteroptera [aq.]	-	-	-	-	1.04	4.34
Homoptera - Afida	-	-	-	-	-	-
Homoptera - Cicadellidae	0.35	3.33	-	-	-	-
Coleoptera - Cantharida	-	-	-	-	-	-
Coleoptera - Carabida	-	-	-	-	2.08	8.69
Coleoptera - Crizomelida	-	-	0.06	2.94	-	-
Coleoptera - Coccinelida	-	-	0.06	2.94	-	-
Coleoptera - Curculionida	1.41	13.33	0.06	2.94	4.16	13.04
Coleoptera - Dytiscida	-	-	0.06	2.94	9.37	30.43
Coleoptera - Dytiscida [L.]	0.70	6.66	0.06	2.94	-	-
Coleoptera - Elaterida	-	-	-	-	-	-
Coleoptera - Scarabeida	0.70	3.33	0.06	2.94	-	-
Coleoptera - Stafilinida	0.35	3.33	0.06	2.94	-	-
Coleoptera - undet.	28.97	80.00	2.33	50.00	52.08	86.96
Panorpata	-	-	-	-	-	-
Lepidoptera	-	-	-	-	-	-
Lepidoptera [L.]	-	-	0.37	14.71	1.04	4.34
Trichoptera	-	-	-	-	-	-
Diptera - Nematocera (Typulida)	1.06	10.00	0.12	5.88	-	-
Diptera - Nematocera (Culicida)	2.12	16.67	0.44	14.71	-	-
Diptera - Nematocera [L.]	35.33	70.00	0.06	2.94	-	-
Diptera - Brahicera	16.96	56.67	5.11	70.59	14.58	34.78
Diptera - Brahicera [L.]	-	-	0.06	2.94	-	-
Hymenoptera-Formicida	9.18	36.67	0.50	11.76	1.04	4.34
Hymenoptere-Apida	-	-	0.06	2.94	-	-
Hymenoptera-undet.	-	-	0.18	8.82	-	-
Amphibia [L.]	-	-	-	-	-	-

Table 2. (Continued)

	Habitat 4		Habitat 5		Habitat 6	
	A%	F%	A%	F%	A%	F%
Anelida	0.48	3.27	0.84	5.00	-	-
Bivalva	-	-	-	-	-	-
Gasteropoda [t.]	0.48	4.91	3.17	2.50	-	-
Gasteropoda [aq.]	-	-	-	-	-	-
Izopoda [t.]	2.12	14.75	0.63	5.00	-	-
Izopoda [aq.]	0.65	6.55	0.63	2.50	-	-
Arachnida - Acaria	0.32	1.63	0.63	7.50	-	-
Arachnida - Araneida	3.42	24.59	2.53	25.00	2.52	20.34
Arachnida - Opiliona	-	-	0.21	2.50	-	-
Arachnida - Pseudoscorpionida	0.16	1.63	0.21	2.50	-	-
Miriapoda - Chilopoda	0.32	3.27	-	-	-	-
Miriapoda - Diplopoda	0.16	1.63	0.63	5.00	-	-
Collembola	1.30	9.83	-	-	0.97	5.08
Ephemeroptera [L.]	0.32	3.27	-	-	-	-
Plecoptera	0.16	1.63	-	-	-	-
Plecoptera [L.]	0.81	6.55	0.21	2.50	-	-
Odonata	-	-	-	-	0.38	3.39
Odonata [L.]	0.16	1.63	-	-	0.19	1.69
Ortoptera	0.16	1.63	0.21	2.50	-	-
Heteroptera [t.]	0.97	8.19	0.84	7.50	1.35	10.17
Heteroptera [aq.]	-	-	0.84	5.00	0.38	3.39
Homoptera - Afida	0.81	4.91	1.69	10.00	26.21	38.98
Homoptera - Cicadellidae	0.32	3.27	1.90	12.50	0.77	5.08
Coleoptera - Cantharida	0.16	1.63	0.42	5.00	0.19	1.69
Coleoptera - Carabida	1.63	11.48	0.63	5.00	0.19	1.69
Coleoptera - Crizomelida	-	-	-	-	0.19	1.69
Coleoptera - Coccinelida	-	-	-	-	-	-
Coleoptera - Curculionida	0.32	3.27	0.21	2.50	1.16	6.78
Coleoptera - Dytiscida	-	-	-	-	-	-
Coleoptera - Dytiscida [L.]	1.14	4.91	-	-	-	-
Coleoptera - Elaterida	0.32	3.27	-	-	0.58	5.08
Coleoptera - Scarabeida	-	-	-	-	-	-
Coleoptera - Stafilinida	1.14	6.55	-	-	0.19	1.69
Coleoptera - undet.	38.00	78.69	48.20	82.50	36.50	76.27
Panorpata	0.16	1.63	-	-	-	-
Lepidoptera	0.48	4.91	-	-	-	-
Lepidoptera [L.]	2.28	19.67	0.42	5.00	2.33	11.86
Trichoptera	0.32	3.27	-	-	0.19	1.69
Diptera - Nematocera (Typulida)	7.17	39.34	1.26	5.00	1.16	6.78
Diptera - Nematocera (Culicida)	2.77	18.03	0.63	5.00	2.13	5.08
Diptera - Nematocera [L.]	1.14	8.19	0.21	2.50	0.58	5.08
Diptera - Brahicera	17.45	72.13	26.42	70.00	5.43	28.81
Diptera - Brahicera [L.]	0.48	4.91	-	-	-	-
Hymenoptera-Formicida	10.44	36.07	5.49	22.50	15.92	33.90
Hymenoptere-Apida	0.32	3.27	-	-	-	-
Hymenoptera-undet.	0.81	8.19	0.84	10.00	0.38	3.39
Amphibia [L.]	0.16	1.63	-	-	-	-

Table 3. The frequency of empty stomachs and the Shannon Weaver index (H)

	Habitat 1	Habitat 2	Habitat 3	Habitat 4	Habitat 5	Habitat 6
The frequency of empty stomachs	-	2.90	8.70	1.60	5.00	5.10
Shannon Weaver index (H)	1.70	0.55	1.77	2.29	1.74	1.87

and smaller in the third one where the Coleopteras and Brahicerases register the maximum abundance.

The minerals appear only in the case of habitat 6, which is found in an abandoned quarry, having a stony substratum and the water loaded with suspension. Their consumption can be regarded as accidental, the minerals not having a nutritious value.

An environmental influence can be noticed regarding the abundance of the prey taxa. The trophic spectrum of the amphibians is a result of the complex interaction between its characteristics and the preys' (Santos et al. 2003). On the other hand, frogs are considered an indicator of the environmental conditions, their feeding also implying the trophic offer (Bellocq et al. 2000). Thus, the presence of more sensible preys, such as Ephemeropteras and Plecopteras, can be observed in habitats that are sheltered from the sunbeams and closer to the woods. These are also indicators of clean water. The habitats that do not present these conditions lack these types of prey, being replaced with other preys, especially terrestrial ones (Formicida, terrestrial Izopoda and Coleoptera).

We can notice certain differences of the abundance of the preys regarding the sexes. According to Naya & Bozinovic (2004) the variations of the trophic spectrum are highly connected to the reproduction period, respectively to the sex. A higher flexibility of the gastrointestinal tract appears in the case of the males, which is connected with the annual feeding cycle, while in the case of the females is more connected to the reproductive cycle (Naya & Bozinovic 2004). The females register a higher abundance of certain preys (terrestrial Coleopterans, adult Dipterans Brahicerases, Collembolans etc), the higher feeding intensity being a result of their high energetic needs in the reproducing period.

We can observe a majority of the smaller sized preys in the case of the males, while the larger ones prevail in that of the females. This fact probably appears as a result of the different hunting strategy. According to the scientific literature, the males usually use the active foraging strategy (Perry & Pianka 1997), practically seeking for the prey, thus having a higher possibility of capturing more diverse preys. Meanwhile, the quantity of necessary food is more important to the females,

which feed with the most accessible preys from the vicinity. Therefore, they use the sit and wait strategy, thus capturing larger-sized preys, and saving energy at the same time. This aspect of the trophic spectrum is important because the way in which a species uses its food resources highly determines its chances of survival (Cuello et al. 2006).

The Diptera Nematocera larvae appear in very high amounts only in habitat 1. Considering that they are invertebrates connected especially to the forestry medium, they are probably more abundant in this habitat, thus representing a potential prey for the frogs of this pond. On the other hand, their presence influences the abundance of the aquatic preys, which is the highest in this habitat in comparison to the other puddles. Meanwhile, the Brahicerases adults, which actually replace the previous ones, register very large amounts in habitat 5. They are probably missing from this habitat because of the unfavorable conditions.

The Formicida appear with very high abundances only in the case of the smaller habitats. This fact suggests that the trophic offer of these puddles is not sufficient to satisfy the energetic needs of the present populations. Therefore, the frogs have to hunt in the terrestrial environment. On the other hand, only the terrestrial preys appear with very high abundances in these puddles (terrestrial Coleoptera, Brahicerases and Homoptera Afida).

Regarding the frequency of prey taxa we can observe a serious difference of a certain taxa towards its abundance value, phenomenon also encountered at other species. Similar to that of the abundance, the Coleopterans register a very high frequency, which indicates that few individuals have not accidentally consumed them in a large number, being more accessible in the habitat. In the case of the other preys, this difference can be explained by their size variations. Thus, the larger ones were consumed more frequently but in a lower number, while the smaller ones were mainly randomly consumed in a larger number because of their lower nutritious content. The Collembolans clearly emphasize this fact, which are consumed in a very high number, but by very few individuals.

We can notice the relatively high number of caterpillars in the case of the juveniles. They have a

higher energetic content (Brooks et al. 1996), respectively can be easily captured and therefore represent a potential food for the juveniles, which need energy for growing.

The very low food diversity of the second habitat is very interesting. The individuals from this habitat consumed the same prey taxa (Collembolans) in a very large number, which decreased the diversity. The relatively high diversity of the third habitat is interesting, despite the low average number of preys. This fact suggests a somewhat selectivity of the toads towards the preys. Because of the larger size of the puddle, the position inside a forest and the relatively anthropically unaffected conditions, the pond displays of a rich invertebrate fauna, as proved by the high diversity of the consumed preys. However, the consumed preys in high abundances are the larger ones, the Brahicerata and Coleoptera, from which the toads do not have to eat in large numbers, these registering a higher nutritious content. Thus, the frogs can coexist in their habitat with a great variety of elements of potential food, their trophic spectrum being influenced by the size of the prey, its mobility and its nutritious value, its accessibility and the abundance of the preys (characteristics of the population, which are locally affected by the physical and ecological conditions) (Stebbins & Cohen 1995, Pough et al. 1998).

Concerning the origin of the prey taxa, we can observe that the majority are terrestrial. The presence in a majority of terrestrial preys can be explained by the fact that the size of the ponds from where the frogs were captured are relatively small, thus the frogs are forced to come out and hunt around the ponds. Beside this fact, the terrestrial insects can play a significant role, which are captured from the surface of the plants from the pond, respectively from the water. In the same time the small and large sized preys are present, which indicates that the frogs do not have a selective feeding, having an opportunistic behaviour, feeding with any prey that is accessible and proper as size for swallowing.

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