

## Comparison between males and females feeding habits from three populations of *Bombina variegata* from Vadu Crișului, Pădurea Craiului Mountains, Romania

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**Abstract.** We studied the feeding habits of males and females *Bombina variegata* from 3 habitats in Vadu Crișului. A number of 178 individuals were analysed. Because of the drought, the theory about the different feeding strategies in males and females can be accepted just in case of one of the studied population. Over 95 % of the prey consumed by the studied individuals is terrestrial. The most frequently consumed prey is Formicida, Coleoptera, Araneida, followed by Gasteropoda and Lepidoptera larvae in one population. Vegetal remains, shed-skin and minerals were found too in the yellow bellied toad stomach content. Both males and females stomachal content was high in vegetal remains.

**Key words:** feeding, *Bombina variegata*, three habitats, Vadu Crișului

### Introduction

By being hypothetically effected by stressors such as acid precipitation, environmental contaminants, the introduction of exotic predators, disease agents, parasites, and the effects of ultraviolet radiation amphibians are great indicators of the ecosystem health (Welsh & Ollivier 1998) playing a unique role in nature. The properties of their skin is one of the motives for their uniqueness among the vertebrates because of its usage in respiration and hydration (Pough 2007). This properties make amphibians sensitive to the environmental toxins (Alford & Richards 1999, Boutilier et al 1992, Maerz et al 2005, Shoemaker et al 1992). At the same time the lack of internal thermoragulation makes possible the turning, of more than 50 %, of prey in their own body mass. This fact makes them less finical to the size of their prey than other vertebrates (Pough 1980, Claussen & Layne 1983), eating whatever moves across their field of view (Lettvin et al 1959).

Studies upon amphibian feeding were made in the past (e.g. Taraščuk 1959, Kuzmin 1990, Cicort-Lucaciu et al 2005, 2007, Covaciu-Marcov 2002, 2005 a, b, Ferenți et al 2007, Sas et al 2005 a, b, c) and scientist will continue with this kind of studies due to the close link between the frogs and toads and the environment and the information that their diet can give us.

We studied the trophic spectrum of 3 *Bombina variegata* populations from Vadu Crișului, Pădurea Craiului Mountains. The reason of choosing this toad is the widespread range that this amphibian occupies in Romania (Cogălniceanu 2000), so that we easily could find different close populations for comparison.

Studies about the feeding habits of the yellow bellied toad, in the area of Pădurea Craiului Mountains, were also made in the past (Sas et al 2005 b, Groza et al 2006).

### Material and methods

The study took place at 3 different habitats in the area of Vadu Crișului. One of the habitat can be found in the close proximity of the village between a railroad and a 100 m high steep cliff (fig. 1) and the other two are situated in a 530 m high forested hill, 3 km away from the first site.

The first site is made by a 75 m<sup>2</sup> pond situated at the same altitude as the village (270 m). The depth of this pond is variable decreasing from near the cliff to it's shore. The moisture and the shadow of the nearby trees made possible the existance of moss all along the shallow shore. Some other specie of amphibian are present here too : *Triturus vulgaris*, *Triturus cristatus*, *Rana dalmatina*, *Rana ridibunda*.

The other 2 habitats, situated 10 m from eachother, are home to a *Bombina variegata* metapopulation. Here we've found *Triturus vulgaris* and *Rana dalmatina* too. One of this habitats is a 45 m<sup>2</sup> pond made from 3 diferent little slops linked toghether by narrow bowls in the ground. The other forest habitat is a 30 m<sup>2</sup> pond situated 1 m lower than the first. The morphology of the two forest ponds is almost the

same: some of their shores are abrupt when others are shallow; their bottoms are covered in a thick mulch; the aquatic vegetation is absent.

We'll refer to the pond nearby the cliff as PNC and to the other two ponds as P1 and P2 (P1 - the pond made from the 3 little slopes and P2 - the other one).

The study was made in July and August 2007. We analyzed a total of 178 individuals : 81 from P1, 50 from P2 and 47 from PNC. They were caught by hand or with the help of long handle nets. We tried to analyse 13 or more individuals / population / day of study. However due to the environmental conditions the minimal number of individuals studied one time at PNC was 11.

The technique used in analyzing the stomachal content of the studied individuals is the stomach-flushing method

(Fraser 1976, Legler & Sullivan 1979, Cogălniceanu 1997). Due to the power of their gastric acid (Caldwell 1996) the stomachal contents were immediately collected from the individuals to avoid the result vitiation. This technique of stomach-flushing involves a syringe with a narrow plastic tube at the end. The tube is introduced in the stomach of each individuals and a stream of water is then pushed into the toad. The stomachal content is placed in airtight tubes and preserved with formaldehyde 4 %.

Every test tube is opened in the laboratory and its content is carefully analyzed with the help of speciality literature (Crisan & Muresan 1999, Crisan & Cupsa 1999, Radu & Radu 1967, 1972).

The goal of the study is a comparative analysis between male and female feeding habits from different habitats.



PNC



P2



P1

Figure no.1 The studied ponds

## Results and discussions

42 categories of animal prey belonging to 20 taxa were found in the collected stomachal contents of the studied individuals. Beside animal prey the toads also ate vegetation, shed-skin and minerals, trophical lifeless objects found in other studied populations of amphibians too (Aszalós et al 2005, Çiçek & Mermer 2006). A review of the scientific literature concerning the feeding habits of

*Bombina variegata* reveals an identical story ( Sas et al 2005 a, c, Groza et al 2006).

Vegetal remains as well as the minerals and shed skin are ingested by mistake together with the live prey (Whitaker et al 1977, Sas et al 2005 b). The positive link between the number of animal prey and the frequency of the vegetation remains supports this theory (fig. 2), the population of P1, being the population with the largest number of animal prey consumed (463). From all the non-

animal trophical objects the vegetation has the highest frequency in the stomachal content of the studied individuals.

Shed skin feeding is present in more than 13 % of the studied individuals. The cannibalism, a widespread behaviour within generalist predators (Polis 1981, Rosenheim et al 1995), could be an explanation for the high frequency of moled skin.

Trying to catch a conspecific individual the toad retains just the moled skin wich is coming off the prey (Sas et al 2004). Examples of cannibalism can be found in other populations of amphibians (Covaciu-Marcov et al 2006, Peter et al 2005) . Another explantion could be protein recycling in Welldon’s opinion (1993).

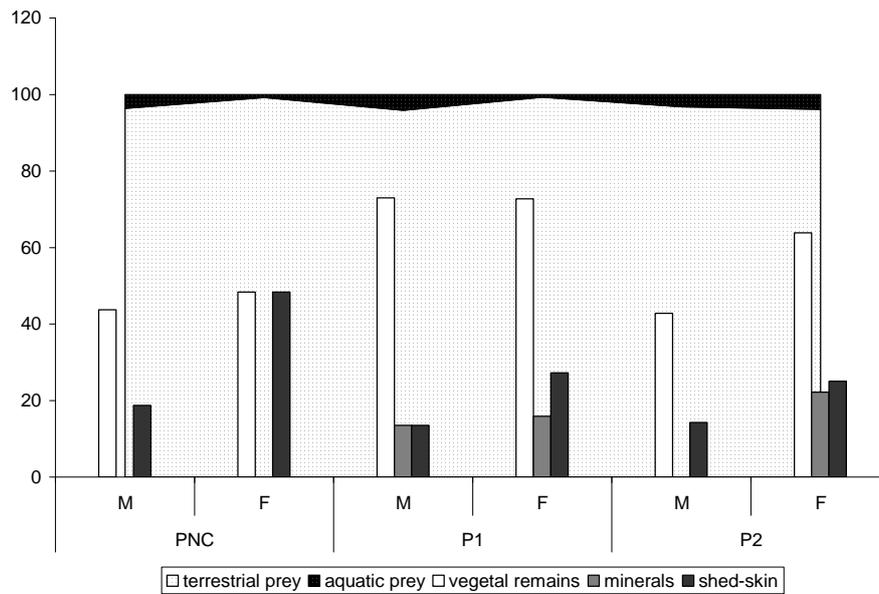


Figure no.2 Terrestrial and aquatic prey; vegetation parts, minerals and shed-skin

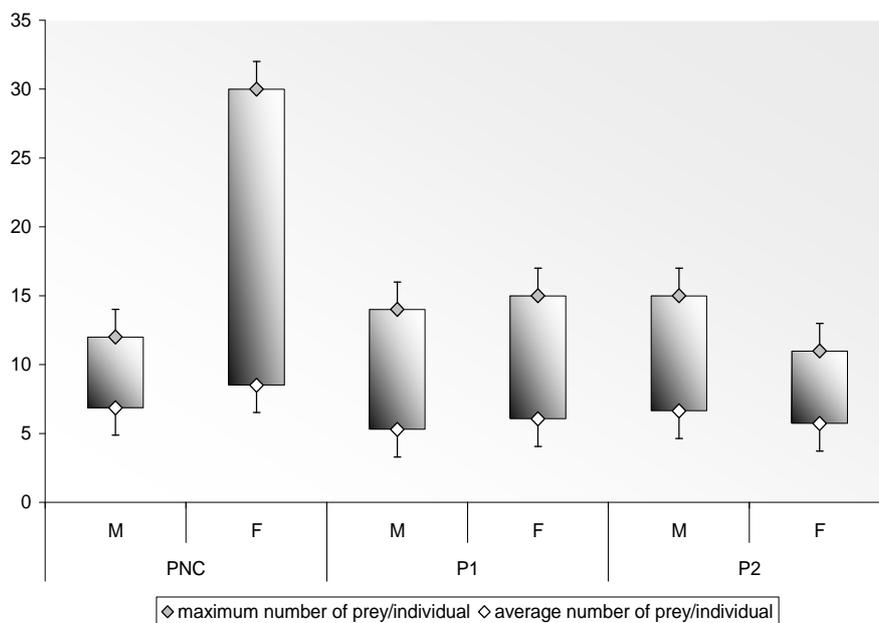


Figure no.3 Maximum and average number of prey / individual

There is a close link between the frequency of non animal food consumed by yellow bellied toad and the intensity of feeding with live prey. Therefore the frequency of vegetal remains, minerals and shed-skin is higher in females.

Minerals are found just in P1 and P2 individuals. However they weren't found in the stomachs of P2 males. A possible explanation is the fact that the individuals of PNC are feeding on the surface of the water, with whatever insect that is unfortunate enough to fall into the water, or in the water mass, where minerals are absent. Small

number of terrestrial Coleopterans, that are generally being found on the surface of the ground, occur in their stomachal contents. Formicida and Gasteropoda were caught next to the pond shore where is not possible to ingest any minerals due to the presence of moss.

Just one case of empty stomach has been found and another individual with nothing more than shed skin inside the stomach. Being given the circumstances this two individuals not feeding is just an accident.

**Tabel no.1** Weight of the most important prey items in males and females individuals

	PNC		P1		P2	
	M	F	M	F	M	F
<i>Gasteropa</i>	14.54	4.16	2.55	0.37	0	0.97
<i>Lamellibranchia</i>	0	0	0	0	1.07	0.97
<i>Izopoda terrestrial</i>	2.72	0.75	0.51	1.12	0	0.48
<i>Isopoda aquatic</i>	3.63	0.37	0	0	0	0
<i>Pseudoscorpionidae</i>	0.9	0.37	0	0.37	2.15	0.48
<i>Acaria</i>	0	1.13	0	0.37	1.07	0.48
<i>Arahnida Araneida</i>	14.54	7.19	9.69	10.86	6.45	14.07
<i>Arahnida Opilionidae</i>	0	0.75	6.12	2.99	2.15	1.45
<i>Diplopoda</i>	0	0	0	0.74	6.45	1.45
<i>Colembola</i>	0.9	0	2.55	7.11	2.15	1.94
<i>Ortoptera</i>	0	1.13	2.04	1.49	3.22	3.39
<i>Dermaptera</i>	0	0	0.51	1.49	0	0
<i>Heteroptera</i>	0.9	2.27	0	0.74	4.3	0.97
<i>Homoptera Afidina</i>	1.81	0	1.02	0	0	0
<i>Homoptera Cicadina</i>	0.9	1.51	1.02	2.99	3.22	0.97
<i>Coleoptera larvae</i>	0.9	0	1.53	0	1.07	1.94
<i>Coleoptera unidentified</i>	1.81	4.54	8.14	5.24	6.45	10.19
<i>Coleoptera Ditiscidae</i>	0	0	0	0	1.07	0
<i>Coleoptera Carabidae</i>	0	0	1.02	0.74	6.45	1.94
<i>Coleoptera Curculionidae</i>	0	0.75	1.53	0.74	0	2.91
<i>Coleoptera Coccinellidae</i>	0.9	0.37	0	0.37	0	0.48
<i>Coleoptera Stafilinidae</i>	0	0.75	1.53	0.37	0	0
<i>Coleoptera Scarabeidae</i>	0	0	1.02	0	2.15	0.96
<i>Coleoptera Elaterida</i>	0	0.37	0.51	0.74	1.07	1.45
<i>Diptera Nematocera larvae</i>	0	0	3.06	0	0	0.97
<i>Dipera Brahicera</i>	1.81	3.4	2.55	0.74	3.22	1.45
<i>Diptera Nematocera</i>	2.72	0.37	1.02	3.74	10.75	5.33
<i>Lepidoptera larvae</i>	14.54	4.92	1.53	3.37	0	3.88
<i>Himenoptera unidentified</i>	2.72	1.13	2.55	0.74	0	1.45
<i>Himenoptera Formicida</i>	31.81	57.19	47.44	49.06	32.25	31.55

Feeding intensity was estimated, calculating the average and maximum number of preys / individual. Small difference between the average and the maximum number of prey / individual as seen in males of PNC and females of P2 marks out a balanced feeding in all of the individuals, eating both large and small prey. The biggest difference between this two values has been recorded in females of PNC because of some individuals feeding with big quantities of small food (Formicida).

Males represent 34,64 % from the total number of studied individuals. From a total of 1136 preys 35,12 % were captured by them. So, generally

speaking, there is a similarity concerning the average number of prey per individual between the sexes.

Invertebrates represent the base feeding object in amphibians (Das 1996). Same feeding habits has been found in the case of our study too.

Formicida, Araneida and Coleoptera have the biggest weights from all of the prey taxa. This 3 taxa represent more than 45 % of the total prey in all 3 analysed populations. A great quantity of snails and caterpillars have been eaten too by the individuals from PNC. This can be a proof of the individuals from here, especially male individuals, preferring a low mobility prey.

**Table no.2** Frequency of apparence of the most important prey items in males and females individuals

	PNC		P1		P2	
	M	F	M	F	M	F
<i>Gasteropa</i>	62.5	19.35	8.1	2.27	0	5.5
<i>Lamellibranchia</i>	0	0	0	0	7.14	2.7
<i>Izopoda terrestrial</i>	18.75	6.45	2.7	6.81	0	2.7
<i>Isopoda aquatic</i>	6.25	3.22	0	0	0	0
<i>Pseudoscorpionidae</i>	6.25	3.22	0	2.27	14.28	2.7
<i>Acaria</i>	0	3.22	0	2.27	7.14	2.7
<i>Arahnida Araneida</i>	50	38.7	32.43	47.72	28.57	50
<i>Arahnida Opilionidae</i>	0	3.22	16.21	13.63	7.14	8.33
<i>Diplopoda</i>	0	0	0	2.27	14.28	8.33
<i>Colembola</i>	6.25	0	5.4	18.18	14.28	8.33
<i>Ortoptera</i>	0	9.67	10.81	9.09	21.42	16.66
<i>Dermaptera</i>	0	0	2.7	9.09	0	0
<i>Heteroptera</i>	6.25	19.35	0	4.54	14.28	5.5
<i>Homoptera Afidina</i>	6.25	0	5.4	0	0	0
<i>Homoptera Cicadina</i>	6.25	12.9	5.4	15.9	14.58	5.5
<i>Coleoptera larvae</i>	6.25	0	8.1	0	7.14	11.11
<i>Coleoptera unidentified</i>	12.5	32.25	35.13	27.27	42.85	41.66
<i>Coleoptera Ditiscidae</i>	0	0	0	0	7.14	0
<i>Coleoptera Carabidae</i>	0	0	5.4	4.54	35.71	11.11
<i>Coleoptera Curculionidae</i>	0	6.45	8.1	4.54	0	8.33
<i>Coleoptera Coccinelidae</i>	6.25	3.22	0	2.27	0	2.7
<i>Coleoptera Stafilinidae</i>	0	6.45	8.1	2.27	0	0
<i>Coleoptera Scarabeidae</i>	0	0	5.4	0	7.14	2.7
<i>Coleoptera Elaterida</i>	0	3.22	2.4	4.54	7.18	8.33
<i>Diptera Nematocera larvae</i>	0	0	2.4	0	0	2.7
<i>Dipera Brahicera</i>	12.5	25.8	13.51	4.54	21.42	8.33
<i>Diptera Nematocera</i>	18.75	3.22	5.4	9.09	28.57	25
<i>Lepidoptera larvae</i>	37.5	25.8	8.1	20.45	0	19.44
<i>Himenoptera unidentified</i>	18.75	9.57	13.51	4.54	0	8.33
<i>Himenoptera Formicida</i>	62.5	87.09	72.97	79.54	64.28	66.66

A possible explanation of feeding with caterpillars is their feeding habits. Leaves from the nearby trees represent a food source for them so accidents of them plummeting into the water can occur easily. Snails are probably attracted to the moist places in a dry season so their presence near the water makes them vulnerable to the toad attacks.

Number of preys depends on the prey body size, smaller prey being eaten in larger amounts than bigger prey. Therefore great number of ants were consumed by the investigated individuals. The frequency of individuals with ant stomachal content is high as well. Studies upon amphibian skin toxins (Daly et al 1994, Spande et al 1999, Saporito et al 2004) assign the presence of alkaloids into their skin to the small leaf litter prey in their diet, particularly ants. Maybe the high amount of Formicida in the yellow bellied toad stomachal content is a good example in this way too, or maybe this is just a result of large number of ants populating the amphibian's habitat.

High frequencies were found in case of feeding with spiders and beetles too. The reason for this is the variety and the large number of Coleoptera and Araneida in the studied habitats.

Other studies, about *Bombina variegata* tropical spectrum, indicate this 3 invertebrates orders as a priority in the yellow bellied toad diet. Great numbers of aquatic crustacians as Amphipoda, Cladocera (Sas et al 2004, Sas et al 2005 a, Groza et al 2006) are being found as well in the analysed stomachal content of those individuals. In the case of this study crustacians do not play an important role in the toads feeding. For a change Gasteropoda and Lepidoptera larvae are being found in great numbers in the stomachs of PNC males. The other individuals, both males and females are feeding with a great variety of food, so besides Formicida, Coleoptera and Araneida, none of the prey items is more important than the others.

Over 95 % of the prey in the stomachal content of the individuals is terrestrial prey. In a way this can be a proof of the toad being more terrestrial than aquatic but another explanation for the high frequency and weight of terrestrial food can be the drought that brought a great variety of animals in the close proximity of the water either for moisture or for a drink. Large numbers of terrestrial prey in *Bombina variegata* were found in other populations too (Sas et al 2004, Groza et al 2006, Peter et al 2007). By analysing results from this studies and comparing with our own results, the second

hypothesis, of the drought influencing the toad menu is sustained.

Even if migrating between ponds there are some differences between the individuals from P1 and P2 concerning their diet. This differences probably occur because of some little differences in the ponds morphology. P2 has a more clear water and a small depth. This is why some of the prey is being easily caught by the individuals here. In addition, the shores of P2 are less abrupt than the shores of P1. This makes easier for the individuals to walk out the water.

The clarity of the water makes available prey like clams, prey from the bottom of the pond, that can't be visible in a dirty water. At the same time this slowly and rarely seen moving animal is not so attractive for the predator. Another difference is the mobility of some of the prey, case in which, in our opinion, individuals from P2 caught more easily high mobile prey due to the shallow shore of their habitat.

Differences in male and female feeding ecology appear in the three studied habitats and in the same habitat as well. In general males are using the "active foraging" method while females are using the "sit-and-wait" technique for prey catching. This feeding strategies are a result of mate searching in males, and egg laying in females. A lot of energy can be preserved by the females for reproductive purposes this way. This general theory, with males feeding with a more slower prey and females with a high mobility one, due to their technique, is compatible just with the case of PNC population. Over 37 % of the males in here consumed Lepidoptera larvae and 62,5 % of them consumed Gasteropoda.

In P1 and P2 populations feeding habits varies. Males and females are feeding with both low and high mobility invertebrates. As an example Orthopterans - high mobility prey - were being eaten mostly by the males in P1 and by the females in P2, Lepidoptera larvae - low mobility prey - are present in high percentages in the stomachs of the females. So in P1 and P2 populations we can't talk about a preference for one kind of food and neither about a different strategy of feeding.

We found little differences concerning the provenience habitat of the most preys. The majority of the prey was terrestrial in both males and females. However small differences occur. Females from PNC and P1 consumed with 4 % more terrestrial prey than the males. Slightly different is the case of P2 where the males

consumed a bigger weight of terrestrial prey. Here the difference between the two is just 0,66 %.

### Conclusions

Just one individual from 178 was found without stomachal content. This can be an indication of the optimal feeding conditions of the toad in the 3 studied habitats.

Along the animal prey we also found vegetal remains, moled skin and minerals, accidental trophical objects, swallowed together with the proposed prey.

As found in others studies on *Bombina variegata* the provenience environmet of the preys found in the stomachs of analised individual is mainly terrestrial. The high percentage of terrestrial prey found in our study is due to the drought that attracts many invertebrates to the close proximity of the water. Little differences were discovered in the ratio of aquatic and terrestrial prey between the sexes.

The most important animal prey items are Formicida, Coleoptera and Araneida, prey found in general in high amounts in the stomachal contents of other populations of *Bombina variegata*.

Differences in prey mobility preference in males and females exist just in the case of PNC habitat. In P1 and P2 there is an equilibrium between high and low mobility prey items preferences. Probably here the preference for one or other prey animals dissappear due to the rarity of feeding opportunities.

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