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Food composition of three *Bombina variegata* populations from Vâlsan River Protected Natural Area (Romania)

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Abstract. Our study aimed to analyse the food composition of three *Bombina variegata* populations from three different habitats from Vâlsan River Protected Natural Area. The most intense feeding and the highest trophic diversity were registered in a slow-flowing aquatic habitat with stagnant sectors, surrounded by different terrestrial habitats. In this case, the toads can feed with both the resources of the populated aquatic habitat and massively hunt in the neighbouring terrestrial areas, this variability assuring a rich amount of preys, both quantitatively as well as qualitatively. The three populations consumed 604 preys belonging to 34 prey taxa categories. Vegetal matter, shed-skin fragments and inorganic elements were also identified in the stomach contents, beside the animal preys. The ants hold high percentage abundance and frequency of occurence values in the case of all populations, underlining their importance for *B. variegata*. Although there are feeding differences between the populations, these are not significant. Our results highlight the importance of preserving the aquatic habitats populated by *B. variegata*, as well as that of the neighbouring terrestrial ones and also of maintaining the access of the toads to them. Practically, the species can adequately feed if it can go back and forth from the aquatic to the terrestrial habitat. In this context, the road works that take place in the protected area do not favour the future of some *B. variegata* populations from Vâlsan River Protected Natural Area.

Keywords: *Bombina variegata*, feeding, prey items, protected area, conservation status.

Introduction

Recent data have indicated the existence of some important differences between the food composition of the yellow bellied toads that occupy neighbouring microhabitats, which are, however, morphologically different (Ferenți et al. 2010a). Moreover, it has also been recently proved that there are some man-made differences in the feeding of some *Bombina variegata* populations, which scarcely feed in the anthropogenic affected habitats (Kovács et al. 2010). The influence of the microhabitats' characteristics and especially the anthropogenic influence are extremely important factors for the amphibians, which have a complex activity, being present both in the aquatic and terrestrial ecosystems. Their feeding is important through the diversity and the quantity of consumed preys, amphibians being consumers both in aquatic and terrestrial habitats (Whiles et al. 2006). Thus, the ecologic characteristics of the consumed preys have demonstrated that some frog species perform movements at certain distances and in different habitats from the populated ones in search of food (Tomescu et al. 2010). This type of data is even more important in the protected areas, almost all of the amphibian species from Romania being protected (OUG 57/2007). From these, *B. variegata* is a species with a high conservative value (OUG 57/2007), although it is relatively common and well represented in the hilly and mountainous areas from Romania. Therefore, there are numerous feeding studies performed in the country (eg. Ghiurcă & Zaharia 2005, Groza et al 2006, Ferenți et al. 2007a, Toth et al. 2007, Dimancea & Covaciu-Marcov 2009, Kovács et al. 2010). However, very few data are available concerning the feeding of the populations situated in protected areas (Ferenți et al. 2007b, 2010a). The present study aims to analyse the food composition of *B. variegata* species from Vâlsan River Protected Natural Area, following the feeding differences of three populations found in different habitats.

Material and methods

Our study was performed in august 2010. On a whole, we analysed 80 *B. variegata* individuals. These were captured directly by hand, being afterwards held in water buckets. The drawing of the stomach contents was realised in maximum one hour after capturing the first individual, amphibians rapidly digesting their preys (Caldwell 1996). The stomach contents were collected using the stomach flushing method (Solé et al. 2005). Afterwards, all of the toads were released in their habitat. The obtained samples were stored in air-tight test tubes, preserved in 4% formaldehyde and afterwards determined in the laboratory at the magnifying glass. The data were statistically processed, calculating the percentage abundance (%A) and the frequency of occurrence (%f). The food diversity was estimated using the Shannon-Wiener index (H) (Shannon-Wiener 1949). We used the Mann-Whitney U-test (Nachar 2008) in order to establish the food differences between the populations from the three analysed habitats.

The studied *B. variegata* samples were captured from three different habitats (Figs 1.). The first one is represented by a typical mountain stream, with a fast flow and rocky substratum, being a left tributary to Vâlsan River. The habitat is surrounded by a common spruce forest, with open areas and a recreation area with some buildings neighbouring the stream's emptying into Vâlsan. The water depth is of maximum 30 cm, pewter being present on the shores. The second habitat is represented by a ditch, which is approximately 30 m long and 1 m wide, being situated on the road margin. The substratum is silty, lacking in its own aquatic vegetation, some pewter being present on the shores. The ditch is bordered on one side by a road and a pasture, and on the other side, by a partially cleared common spruce forest. The habitat is presently strongly affected by man, the road being re-modified and the ditches from its margins being amended and drained. The third habitat is represented by a relatively wide boggy area, situated between a road and a slope covered by a common spruce forest. The water depth can reach 1 m, the substratum being covered with silt. The vegetation of the bogging area is very abundant, pewter being present on the shores, while in the water, reed, algae and aquatic cormophytes, which occupy much of the water surface.

Figure 1. The three studied habitat (the stream, the ditch and the bog)

Results

All of the 80 *B. variegata* studied individuals presented stomach contents. However, in each of the 3 habitats an individual was identified that consumed only vegetal matter. The lowest feeding diversity was registered in the stream (H=1.78), while the highest (H=2.45) in the ditch (Table 1). The difference between these habitats is also valid in the case of the number of consumed prey taxa, the maximum and average number of prey/individual, these parameters registering the lowest values in the case of the population from the stream and the highest in the one from the ditch. Despite these differences between the feeding of the populations from the three habitats, these variations are not significant in either of the cases, in all of the situations U surpassing 0.05. Thus, between the population from the bog and the one from the stream U=0.70, between the one from the bog and the one from the ditch U=0.51, and between the one from the stream and the one from the ditch U=0.19.

The consumption of mineral, vegetal and shed-skin presents differences between the three habitats. The terrestrial preys dominated the feeding of all three populations (Table 1). However, their amount differs depending on the habitat. The lowest value of the aquatic preys is recorded in the bog (only 0.92 %), while the highest in the ditch (33.08 %).

On a whole, all of the three populations consumed a number of 34 prey taxa categories (Table 2). The exact number of prey taxa is smaller, due to the fact that the larvae forms of the prey taxa were separated from the adults, in some cases, on the basis that they originate from different mediums (the adults being terrestrial and the larvae aquatic), and therefore represent preys that require other capturing methods. From the 34 prey taxa, only 8 were consumed in all of the three habitats. These were terrestrial forms, common in all of the forested areas, or common flying forms, easily accessible in all of the three areas. In all of the habitats, the ants occupy first place regarding the frequency of occurence, being the prey taxa consumed by most of the studied *B. variegata* individuals. The situation is also confirmed in the case of the amount in two of the habitats (stream and bog), while in the third habitat, the ants occupy only second place, at a small distance from the first position. The same prey taxa occupy first places regarding both the frequency of occurence and percentage abundance in the case of all three habitats.

Discussions

The trophic offer from the three studied habitats seems to generally satisfy the trophic necessities of the studied populations. The fact can be deduced from the absence of animals without stomach contents, a trophic spectrum parameter that has been regarded as indicating optimum feeding conditions (Sas et al. 2009). Meanwhile, it can be considered that the time of study is a proper one for the feeding of the species. Generally, amphibians feed intensely at the end of summer and autumn, fact that has also been pointed out in other feeding studies of different species (Covaciu-Marcov et al. 2003, Kovács et al. 2007). On the contrary, feeding is scarce at the beginning of spring, due to the improper thermal regime (Hirai & Matsui 2000, Covaciu-Marcov et al. 2010a,b). During the period and in the studied area, the thermal regime was favourable for the amphibians' feeding, while the positioning in a mountainous area supported an adequate hydric regime.

However, despite the general satisfaction of the trophic necessity of the three populations, there are certain feeding differences between them. Thus, the less intense feeding was registered in the case of the stream, while the most intense one in the ditch. The fact is a consequence of the features of the two habitats. The stream is a restrictive habitat, while the ditch offers more diverse hunting opportunities and automatically higher possibilities of satisfying the trophic necessity. Steep, forested slopes surround the stream. Thus, the ecologic diversity and the trophic resource available to the

toads are reduced. On the contrary, the toads can easily capture aquatic preys from the ditch, stagnant water, being in a close connection with them, but also have access to the terrestrial preys from the neighbouring hay-field and forest. The population from the ditch has at least one additional type of habitat that can be trophically exploited in comparison to the population from the stream. Having a wider hunting territory, the toads from the ditch present higher prey diversity. The species is advantaged in this situation by the fact that it is capable to realise relatively long movements in the terrestrial medium (Hartel 2009, Ferenţi et al. 2010b). The trophic advantage of populating stagnant water or a poor flowing one is also highlighted by the large consumption of Nematocera larvae by the population from the ditch. This prey type completely lacks from the food of the population from the stream. The Nematocera larvae were also massively consumed by other *B. variegata* populations (Ferenţi et al. 2007b, Toth et al. 2007), but lacked from the food of some populations present in streams (Hodişan et al. 2009, Kovács et al. 2010).

Table 1. The number of studied individuals, the maximum and average number of preys/individual, the consumption frequency of vegetal, shed-skin and mineral elements, the number of consumed prey taxa, the amount of aquatic and terrestrial preys and the food diversity.

	Habitats		
	Stream	Ditch	Bog
No. of studied individuals	20	30	30
Maximum no. of preys/individual	15	19	17
Average no. of preys / individual	5.9	8.96	7.23
The consumption frequency of vegetal	65	63.33	53.33
The consumption frequency of mineral elements	5	20	23.33
The consumption frequency of shed-skin	10	30	23.33
No. of consumed prey taxa	22	24	19
% aquatic preys	26.27	33.08	0.92
% terrestrial preys	73.72	66.91	99.07
Food diversity (H)	2.37	2.45	1.78

Table 2. Percentage abundance (A%) and frequency of occurence (f%) of the prey taxa [L – larvae].

	Habitats					
	Stream		Ditch		Bog	
Prey taxa	A%	f%	A%	f%	A%	f%
Nematoda	-	-	0.74	6.66	-	-
Nematomorpha - Gordius	-	-	1.48	13.33	-	-
Oligocheta - Lumbricida	0.84	5	0.37	3.33	-	-
Gasteropoda (snails)	1.69	5	3.71	20	5.52	26.66
Gasteropoda (limax)	0.84	5	-	-	-	-
Gastropoda (*Planorbis* sp.)	-	-	-	-	0.46	3.33
Crustacea - Isopoda	1.69	10	1.11	10	-	-
Crustacea - Gamarida	3.38	5	4.08	30	-	-
Arahnida - Acaria	0.84	5	1.11	10	-	-
Arahnida - Pseudoscorpionida	-	-	-	-	0.92	3.33
Arahnida - Araneida	6.77	5	2.23	16.66	5.99	40
Miriapoda - Diplopoda	4.23	20	2.97	23.33	1.84	13.33
Miriapoda - Chilopoda	2.54	5	-	-	-	-
Colembola	-	-	2.23	10	1.84	10
Plecoptera	-	-	0.37	3.33	-	-
Plecoptera [L.]	20.33	50	3.34	20	-	-
Orthoptera	0.84	5	-	-	1.84	13.33
Coleoptera (undet.)	5.08	20	4.46	36.66	2.76	16.66
Coleoptera - Dytiscidae [L.]	2.54	15	0.37	3.33	-	-
Coleoptera - Elateridae	0.84	5	-	-	0.46	3.33
Coleoptera - Curculionidae	2.54	15	-	-	0.46	3.33
Coleoptera - Stafilinidae	0.84	5	0.37	3.33	-	-
Coleoptera - Carabidae	0.84	5	-	-	-	-
Trihoptera [L.]	-	-	0.37	3.33	-	-
Trihoptera	-	-	-	-	0.46	3.33
Heteroptera	-	-	1.48	13.33	0.92	6.66
Homoptera - Cicadelllidae	2.54	10	4.08	30	6.91	33.33
Lepidoptera [L.]	-	-	1.48	10	1.38	6.66
Diptera - Brahicera	4.23	15	4.08	33.33	6.91	23.33
Diptera - Brahicera [L.]	-	-	1.11	10	2.30	13.33
Diptera - Nematocera	2.54	10	15.24	60	2.76	20
Diptera - Nematocera [L.]	-	-	23.04	70	0.46	3.33
Diptera - Nematocera - Tipulidae	2.54	10	-	-	-	-
Hymenoptera - Formicidae	31.35	60	20.07	63.30	55.76	96.66

Surprisingly, the population from the bog has a scarcer feeding, at least apparently, in comparison to the other two habitats. Thus, the most reduced trophic diversity is registered here (Table 1). The value of the feeding diversity is even more reduced in comparison to other *B. variegata* populations from other areas (Kovács et al. 2010). However, the low value of the trophic diversity in the case of this population must not be regarded as an indicator of not satisfying the trophic necessities of this population, but only a consequence of a consumption based on a certain type or types of preys. Thus, the values from the bog regarding the maximum and average number of preys/individual are higher than in the case of the population from the stream and closer to the one from the ditch (Table 1). These values are higher than in the case of other populations from different regions (Hodişan et al. 2009, Kovács et al. 2010). Thus, the population from the bog consumes a lot, but eats homogenously. It remains to establish whether this homogenous feeding, therefore with a low number of consumed preys in a large quantity, is a consequence of a reduced trophic offer, or of a trophic selectivity of the toads towards certain trophic categories that were abundantly present in the hunting territory. The fewest prey taxa were consumed in the bog, fact obviously responsible for the low feeding diversity. Some prey taxa, such as Gammarida lack from the food of the population, being difficult to be captured from a bog with an abundant vegetation and deeper water, while others, such as worms are probably accidentally missing. Meanwhile, the Plecoptera larvae lack from the stomach contents of the toads from the bog, due to the preference for flowing, well-oxygenated waters.

Meanwhile, the first most important prey taxon, the ants, registers the highest percentage abundance and frequency of occurence of consumption values in the bog. Thus, in this habitat, 55.76% from the total preys is represented by ants, which were consumed by 96.66% of the *B. variegata* individuals. The size of these values becomes even more relevant by comparing them with the prey taxon that occupied second place, representing only 6.91% from the total preys and being consumed by 33.33% of the toads (Table 2). Thus, the fact that so many individuals from a single prey taxon were consumed justifies the low feeding diversity. Moreover, at the boundary between the forest and the populated bog there were present two anthills, which probably constitute the source of the consumed ants. Meanwhile, the selective consumption of these preys must not be excluded. The massive consumption of ants was also recorded in the case of other *B. variegata* populations (Peter et al. 2006, Groza et al. 2008, Kovács et al. 2010). The situation can be explained through the fact that *B. variegata*, a venomous species, uses the consumed ants at generating its venom, fact previously indicated in the case of amphibians (Jones et al. 1999). The consumption of ants was also observed in the situation of other venomous amphibian species (Covaciu-Marcov et al. 2010c, Dimancea et al. 2010). In addition, an obvious selectivity of the species towards ants was recently recorded, by comparing it with another amphibian species that populated the same habitat (Ferenţi & Covaciu-Marcov in preparation). It is difficult to appreciate in our case, whether the bog population selectively consumes ants, or it is about a trophic opportunism eased by the neighbouring of anteaters. However, previous scientific data concerning the ant consumption by *B. variegata* (Bisa et al. 2007, Groza et al. 2008) at least entitle the affirmation of the advantage of feeding with ants in order to produce venom.

The consumption frequency of the vegetal parts is relatively close between the three habitats, however the highest values being registered in the habitat with the most intense feeding, respectively the ditch. This result can strengthen the point of view according to which vegetal fragments are accidentally consumed by amphibians (Whitaker et al. 1977, Solé & Pelz 2007). Thus, their frequency of occurrence increases once with the increase of the feeding intensity (Aszalós et al. 2005, Kovács et al. 2007). As a result, the toads will have more opportunities to swallow vegetal parts as they consume larger quantities of preys. Meanwhile, the present data regarding the consumption of mineral elements seem to underline the idea of their accidental consumption (Cicort-Lucaciu et al, 2005, Lezău et al. 2010). Therefore, the consumption of inorganic material has the highest values in the habitat where the terrestrial preys dominate, such as ants, small-sized preys, which ease the accidental swallowing of mineral elements together with them. Concerning the shed-skin consumption, this records the highest value in the ditch, the habitat with the most intense feeding. The fact proves that in this case, period or at least in these habitats, the rule of the increase of shed-skin consumption in habitats with low trophic offer is not confirmed (Covaciu-Marcov et al. 2010a). It is obvious that, at least in this case, shed-skin is consumed in the same manner as other trophic elements, every time they are encountered, the highest values being registered by the population with the highest feeding activity. It can not be excluded the fact that the low value of shed-skin consumption, in the case of the stream population, could represent a consequence of the characteristics of the respective habitat, where, due to the fast flow, the water quickly moves the shed-skin, the toads not having the possibility to consume them.

Our data indicate that the most favourable habitat for *B. variegata*, concerning the trophic activity is the one with stagnant or slow flowing waters, with low depth, surrounded by a high diversity of terrestrial habitats. Thus, in this variety of habitats with different local conditions, the trophic offer available to the toads is greatly diversified, both quantitatively and qualitatively. The feeding superiority of the species from small-sized aquatic habitats, such as temporary puddles from forested areas, surrounded by terrestrial habitats, suitable for hunting, has also been previously highlighted (Kovács et al. 2010). The fact is a consequence of the life style of the species, which, although is connected to water, mostly moves in the terrestrial medium (Fuhn 1970, Hartel 2008). Thus, despite the fact that it is present in the water, the species mostly hunts in the terrestrial medium, fact highlighted by the major consumption of terrestrial preys, which has also been recorded in numerous studies (Ghiurcă & Zaharia 2005, Hodişan et al. 2009). The situation is also available in the present case, being pointed out the consumption of Diplopoda, Orthoptera, Cicada, etc., preys that are consumed from the pastures or forests from the neighbouring aquatic habitat. Moreover, ants, important preys for the

toads, also originate from the neighbouring terrestrial habitats. These results indicate once more the importance of the neighbouring terrestrial habitat for the feeding of the toads. A similar result was recently indicated in a wide study performed on several *B. variegata* populations from the south-western part of Romania (Ferenţi et al. 2010a). A poorer feeding is obvious in the stream habitat represented by a more difficult aquatic habitat for capturing preys and surrounded by a homogenous and slightly inaccessible terrestrial area. In the case of the ditch, its sectors with stagnant water and shallow depth are similar to the temporary puddles characteristic to the species. In this type of aquatic habitat, the species is also capable of consuming a larger number of aquatic preys, fact highlighted by the increase in their amount.

The absence of important differences between the feeding of the three populations indicates the presence of a homogenous trophic offer in the entire region. The fact can also be deduced from the quantitative importance of the 8 prey taxa that were consumed in all of the three habitats. Thus, these 8 taxa have high percentage abundance and frequencies of occurence, being represented by common preys, abundant and easy to capture. As a consequence, the feeding of the three populations is generally determined by the trophic offer present in the occupied territory. The habitats of each population influence only on a local, smaller scale the feeding of each population. Thus, it is very important not to interfere in the general biodiversity of the protected area, in order to allow the survival of these populations.

Although the studied populations are located in a natural protected area, they are subjected to anthropogenic impact. This artificial influence is manifested through the activities of the tourists, which leave behind mountains of garbage and also through clearcuts. However, the strongest impact for *B. variegata* is presently connected to the modernisation of the road, which crosses the protected area parallel with Vâlsan River. Thus, the ditches from the road margins will also be modified in the area. The fact can have important consequences upon the *B. variegata* populations, which greatly use these ditches. The situation is serious not only by the fact that these populations will be directly decimated, but also that once with the regularization and the straightening of the margins of the ditches, the toads will no longer be able to leave the ditch to hunt in the neighbouring terrestrial habitats, which represent exactly the suitable area for feeding. This fact will have a direct impact in reducing the feeding, which will affect on a longer period the respective populations, these types of situations being previously signalled (Kovács et al. 2010). These consequences are even more serious in a protected area and in the case of a species, which such a high conservative value as *B. variegata* (OUG 57 / 2007).

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