

RESEARCH REGARDING THE YELLOW-BELLIED TOAD, *BOMBINA VARIEGATA*, FROM LEREȘTI AREA, ARGEȘ COUNTY, ROMANIA

NICOLETA DIMANCEA, SEVERUS-DANIEL COVACIU-MARCOV

Abstract. *The present study follows the determination of two aspects regarding a Bombina variegata-like population from Lerești locality, Argeș County. These two directions of analysis aim to establish the trophic spectrum and the affiliation of this population. The toads used very efficiently the food resources from the habitat. Thus, the stomach samples highlighted an overwhelming majority of larvae, respectively those of Nematocera and Ephemeroptera, which are an energetic source easy to obtain and rich from a trophic aspect. The aquatic preys register a majority, being in a direct relation with these data. Moreover, the two grids that define the affiliation of the population emphasized the existence of a typical Bombina variegata-like population. This aspect is in relation with the high altitude of the area.*

Keywords: *Bombina variegata, trophic spectrum, population affiliation.*

Rezumat. *Cercetări privind izvorășul cu burtă galbenă, Bombina variegata, din zona Lerești, județul Argeș, România. Studiul de față urmărește cunoașterea a două aspecte cu privire la o populație de Bombina variegata-like din localitatea Lerești, județul Argeș. Aceste două direcții de analiză vizează determinarea spectrului trofic și respectiv stabilirea apartenenței acestei populații. Populația de buhai s-a folosit foarte eficient de resursele de hrană din habitat. Astfel probele stomacale au evidențiat o majoritate covârșitoare a larvelor, respectiv cele de nematocere și efemeroptere, acestea fiind o sursă energetică ușor accesibilă și bogată din punct de vedere trofic. În relație directă cu aceste date se află și prezența majoritară a prăzilor acvatice. Totodată, cele două grile care conturează apartenența populației au evidențiat existența unei populații tipice de Bombina variegata-like. Acest lucru este în concordanță cu altitudinea mare a zonei.*

Cuvinte cheie: *Bombina variegata, spectru trofic, apartenența populației.*

INTRODUCTION

The two European species of the *Bombina* gender, respectively *Bombina bombina* and *Bombina variegata* are distinct through various aspects, such as morphology, anatomy, ecology and behavior (FUHN, 1960). Thus, the fire-bellied toad, *Bombina bombina* (LINNAEUS, 1761) has adapted to the lower, field areas, preferring altitudes up to 150m (MADEJ, 1973), whereas the yellow-bellied toad, *Bombina variegata* (LINNAEUS, 1758) has characters specific to the higher, hilly and mountainous areas (COGĂLNICEANU et al., 2000). Although they are different because of the distinct areas that they ecologically occupy, however there is an overlapping of the areas, therefore creating a hybridizing zone between the two species (SZYMURA, 1993).

In order to successfully protect a species, it is necessary to know certain aspects regarding its development parameters, such as the way of feeding. At the same time, the existence, or on the contrary the lack of a trophic diversity can suggest certain connections between these aspects and the quality of the environment (INGER & COLWELL, 1977). The majority of the amphibian species from Romania is nationally protected, therefore an expansion of the data regarding their habitats, trophic spectrum, ecological limits of development is required, as well as other information which could contribute to their protection. Studies such as the feeding ones realized by different researchers (SAS et al., 2005, FERENȚI et al., 2007, COVACIU-MARCOV et al., 2004) are of great importance in the accomplishment of this fact. Moreover, *Bombina variegata*-like populations are signalled beginning with low altitudes of approximately 120-350m (COVACIU-MARCOV et al., 2001, 2002) in the western part of the country, at the contact area of the hills with the plain, where the aspect of the habitat allows the existence of these populations.

Therefore, the objectives of this study aim at the following aspects: 1. the establishment of the affiliation of the population at one of the two species; 2. the determination of the trophic spectrum and the outlining of some connections within the trophic elements.

MATERIALS AND METHODS

Our observations were made in august 2008, when we investigated a *Bombina* population composed of 50 individuals (13 females, 37 males) from Lerești locality, Argeș County. The habitat lies in the SE part of the Iezer Mountains at 773 m altitude. The biotope comprises a system of two puddles, being strongly influenced by man. Thus, it is situated between electric pillars and the secondary road 734, which leads to Voina chalet. At the same time, the domestic animals come here and drink water. Moreover, remains of construction materials could be found near the pond at the time of study. Therefore, populations like this one can become vulnerable, especially if they are found within or near a locality.

The analysed habitat is formed of two ponds of different sizes, in a whole covering approximately 5 m wide and 15 m long. The vegetation is very rich, being formed of submersed and immersed species. This aspect encourages

the appearance of invertebrates. The substratum of the pond is formed from a layer of silt, therefore the yellow-bellied toad finds refuge in case of danger.

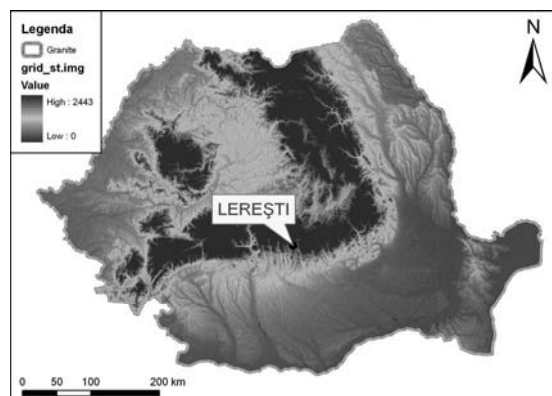


Figure 1. The location of the studied population.

Figura 1. Localizarea populației studiate.

The analysis of the trophic spectrum

The toads were captured using the limnological dredger, and afterwards they were released in their habitat in order to reduce as much as possible the influence of our study. We used the stomach flushing method in order to draw the samples. This method is recommended because of the low rate of injured animals (SOLÉ et al., 2005). This aspect is very important, mainly for the protected amphibians, such as *Bombina variegata*, which is a Natura 2000 species, the method allowing the study of the toads without endangering the status of the species. The stomach contents were stored in airtight test tubes, and afterwards determined in the laboratory using the scientific literature (RADU & RADU, 1967; CRIȘAN & MUREȘAN, 1999; CRIȘAN & CUPȘA, 1999; IONESCU & LĂCĂTUȘU, 1971) and the microscope. The results were statistically processed, following the weight and the frequency of the prey taxa, their origin as well as the relation between these elements.

The analysis of the *Bombina* population affiliation

The study of this aspect was realised in concordance with two grids (the first one was worked out by Szymura & Gollmann, and the second one by STUGREN, 1980), each containing 10 characters which emphasize the chromatic and morphological characters of the two species (SZYMURA, 1993). The grids contain specific features for the parental species, respectively that aspect which is typical for *Bombina bombina* will be graded with 0, and that one that is standard for *Bombina variegata* will be marked with 1. Therefore each individual can raise a score that varies between 0 (for a pure *B. bombina* individual) and 10 (for a pure *B. variegata* sample). The individuals that gathered a score between 2-3 are *B. bombina*-like, between 4-6 are hybrids, and between 7-8 are *B. variegata*-like. These results can be transformed in percentage data and afterwards, the general affiliation of the entire population can be determined.

The first grid analyses 10 chromatic characters regarding the morphology, dimensions and ratio of the light ventral spots. They are red ones at *B. bombina* and yellow at *B. variegata* (FUHN, 1969). A majority holds the black pigmy in the case of the first species, in which isolated islands of light pigment appear. Concerning the second species, the yellow pigment prevails, at the level of which black spots appear accidentally. If the black pigment separates the light spots then the character is specific for *B. bombina*, and if the spots are united then the character is a *B. variegata* one.

Moreover, two aspects have been analyzed regarding the results of the two grids. Thus an average has been made both vertically (in order to determine the percentage of *B. variegata* in each individual) and horizontally (so that to realize a general situation of the characters expressed within the whole population).

RESULTS AND DISCUSSIONS

The analysis of the trophic spectrum

Within the trophic niche, the amphibians are secondary consumers, therefore their diet is primarily composed of animal preys. The identified elements in the stomach contents of the studied population are of animal, vegetal, inorganic nature and respectively shed-skin remains. Within this population there is a strong interrelation between the most important element of the trophic spectrum and the prey origin. Therefore a valuable connexion is set between the high weight of the larvae preys and the mainly aquatic origin of them. This fact suggests that the trophic offer was overwhelmingly rich in larvae forms, thus the toad population did not have to leave the aquatic medium and hunt in the terrestrial one. Because of the different environmental conditions (drought, the lack of an aquatic trophic variety) other

Bombina populations left the aquatic medium, thus the weight of the preys is mainly terrestrial (GROZA et al., 2008, SAS et al., 2004).

From 50 individuals, two samples did not present animal content, registering a relatively low percentage (4%) within the population. Thus, one of the individuals consumed only vegetal matter, while the second one ingested exclusively shed-skin fragments. These cases can be considered as accidental. However, on a whole the weight of the stomachs with content was 100%, thus it can be insinuated that optimum feeding conditions were present. This situation is not a general one, populations that had very high weights of empty stomachs being signalled in the scientific literature (SAS et al., 2003). The maximum number of preys was determined at a male that consumed 28 preys, while the average one was established at 10 preys/individual. Therefore, despite the anthropic factor, the ecological conditions of the pond managed to successfully sustain life development.

On the one hand, the low consumption of minerals (2%) suggests the fact that these were accidentally ingested (SAS et al., 2003, GROZA et al., 2008). On the other hand, the shed skin consumption has higher values, respectively 12%, appearing even an individual that consumed only this type of element. The value is higher in the case of the males, therefore 13.51% of them consumed this element. The relatively high ratio of this type of food can be attributed to epidermal recycling (WELDON et al., 1993), but usually it is considered that it was accidentally swallowed (SAS et al., 2005, FERENȚI et al., 2007).

Regarding the stomach frequency with vegetals, more than half of the individuals (62%) presented this type of content. The most plausible explanation is given by the rich presence of vegetation in this habitat. They do not represent a substantial food element, being accidentally ingested together with the prey (WHITAKER et al., 1977). Generally, the rate is relatively high in comparison to the other elements of the trophic spectrum (shed skin and minerals), cases when their frequency exceeded 80% have been reported (SAS et al., 2005).

In direct relation to these data is the prey origin, which is mainly aquatic (87.60%). Thus, because of the favourable conditions that allowed the persistence of the aquatic habitat, the toads are not forced to leave the medium, the preys being at their hand.

Considering the fact that the amphibians are predators (COGĂLNICEANU et al., 2000), the majority of their diet is composed of animal prey. Thus, within the studied population, the samples revealed a total number of 518 preys. These are composed of invertebrates, from which 98.65% are grouped in the Insect class. The Nematocera (46.20%), respectively the Ephemeroptera (37.40%) larvae, held the biggest weight. Because of the large presence of Ephemeroptera larvae, which are an indicator for clean water, it can be deduced that the respective environment provides healthy development conditions, despite the obvious influence of man. The overwhelming majority of these larvae (together accumulating over 80% from the total prey amount) suggests that the toads had good feeding conditions within the aquatic medium. Therefore, although *B. variegata* is a more terrestrial species than *B. bombina*, still due to the rich trophic offer, they left the aquatic biotope only rarely. The same fact can also be observed at a hybrid *Bombina* population from Oradea, when in August the Nematocera larvae registered very high weights (SAS et al., 2005). At the same time, the larvae stage of the insects is considered to be richer in lipid, and therefore have a higher nutritional content (REDFORD & DOREA, 1984). Regarding the difference between the sexes, the variations are very low, thus they are preferred by both of them due to their accessibility.

The following prey categories are consumed in very low quantities, the majority being swallowed just once by only one individual, thus their value in the trophic spectrum is negligible (0.20%). For this reason, it can be deduced that they were accidentally consumed. Within these low weights, the Formicidae and aquatic Heteroptera register high values, being clearly distinct from the rest of the preys. The ants are small, gregarious animals, therefore they can be consumed in larger quantities and by many individuals. These are important preys for the toads because they represent an easily captured prey, thus they do not make a high effort, maintaining their energy for the large-sized preys. The same situation can be observed at the hybrid population, where the Formicidae occupy second place after the Nematocera larvae, representing approximately 4% of the total consumed preys (SAS et al., 2005).

Within this population, almost the same preys occupy first places in the case of the frequency with which they were consumed. This constancy in the high weight of certain prey taxa and the high number of toads that fed with these preys suggests a homogenous trophic spectrum. Therefore, although *Bombina variegata* lives in reduced aquatic sectors, being thus constrained to search for food in the terrestrial medium, however here at approximately 750m altitude it manages to find optimum conditions so that it does not have to leave its medium. Analyzing the data, it can be noticed that the Nematocera and Ephemeroptera larvae register very high values, therefore they have been consumed due to their richness and high accessibility. Thus, these preys have not been accidentally swallowed in large numbers by few individuals, 86% from the total of 50 individuals have consumed the larvae of these insects. This connection between the frequency and weight of certain taxa is very important, suggesting the toads' opportunistic behavior. Through the fact that it takes advantage of this rich offer, it preserves its energy and on a whole it increases its chances of survival.

However, there are preys that were consumed in a small number by the individuals, but register a relatively high frequency. Thus, the Coleopterans, because of their larger size were consumed in lower quantities by 16% of the toad population. Therefore, it can be observed the presence of preys of different sizes, both small and large, which suggests the fact that the toads do not hunt selectively, but regarding the prey that comes in sight and has the proper size

to be swallowed (ZIMKA, 1966). The fact that the toads capture the preys without preference has been signalled in other feeding studies as well (SAS et al., 2003, FERENȚI et al., 2007).

Table 1. The stomach frequency with vegetals, shed-skin and minerals, the amount (A%) and frequency (F%) of the preys; the origin of the prey taxa.

Tabel 1. Frecvența stomacurilor cu vegetale, exuvie și minerale, ponderea (A%) și frecvența (F%) prăzilor, proveniența taxonilor pradă.

	A (%)			F (%)		
	F	M	T	F	M	T
Vegetals				77	56.76	62
Minerals				-	2.70	2
Shed-skin				7.69	13.51	12
Gasteropoda-snail	-	0.26	0.20	-	2.70	2.00
Gasteropoda-Limax	-	0.26	0.20	-	2.70	2.00
Arahnida-Araneida	-	1.02	0.80	-	10.81	8.00
Miriapoda-Diplopoda	0.92	-	0.20	7.69	-	2.00
Collembola	-	0.26	0.20	-	2.70	2.00
Ephemeroptera (L.)	42.20	36.06	37.40	53.85	56.75	56.00
Odonata (L.)	0.92	0.26	0.40	7.69	2.70	4.00
Homoptera-Afidina	-	0.26	0.20	7.69	2.70	4.00
Homoptera-Cicadina	-	1.02	0.80	-	8.10	6.00
Heteroptera (aq.)	-	4.09	3.20	-	16.21	12.00
Heteroptera	-	1.28	1.00	-	13.51	10.00
Coleoptera	-	1.53	1.20	15.38	16.21	16.00
Coleoptera-Carabidae	-	0.77	0.60	-	5.40	4.00
Coleoptera-Cantharidae	-	0.26	0.20	-	2.70	2.00
Coleoptera-Dytiscidae (L.)	-	0.26	0.20	-	2.70	2.00
Diptera-Nematocera	-	0.26	0.20	-	2.70	2.00
Diptera-Nematocera (L.)	44.95	46.55	46.20	84.62	81.08	82.00
Diptera-Nemat. Typulidae	1.83	-	0.40	7.69	-	2.00
Diptera-Brahicera	1.83	1.28	1.40	15.38	13.51	14.00
Diptera-Brahicera (L.)	0.92	-	0.20	7.69	-	2.00
Himenoptera	1.83	0.77	1.00	15.38	5.40	8.00
Himenoptera-Formicida	4.59	3.58	3.80	23.07	27.02	26.00
Terrestrial	11.01	12.79	12.4			
Aquatic	88.99	87.21	87.6			

The analysis of the population affiliation

The individuals were analysed according to two grids, taken from scientific literature. The studied characters within the grids proved that the population taken into discussion has aspects mainly from *B. variegata*. This fact is determined both by the specific altitude for this species and the habitat's morphology, which influences the type of toad that is found (MACCALLUM et al., 1998). Thus, in some cases, the aspect of the biotope can be even more valuable than the altitude at which it is found. In this respect, populations of *B. variegata*-like have been studied at much lower altitudes in comparison to the studied population (COVACIU MARCOV et al., 2001). In the case of the *B. variegata*-like populations, they reach even 150-160m in Oradea area, because of the fact that in these contact sectors between hills and plains, suitable habitats can be found for this species.

No pure *B. variegata* individual was identified within the population. Thus, three had a close value to that of the hybrids between the two species (respectively 57.5%), 45 individuals were closer to *B. variegata*-like (registering values between 65%-87.5%) while two samples had 90% *B. variegata* characters. On a whole, the population is a typical *B. variegata*-like, having an average of the grids of 75.1%. This situation can also be observed at altitudes of approximately 155m, within the populations from Oradea area, which are also closer to *B. variegata* (COVACIU MARCOV et al., 2001). In comparison, a study from Ier plain upon several hybrid populations identified a reduced percentage of *B. variegata* characters (generally under 10%) at altitudes of approximately 120-130m (COVACIU MARCOV et al., 2002). This fact is due both to the low altitude, type of present relief (field) and the biotope's morphology (large and deep puddles). In the case of the Leresti population, there is not an individual that approaches the fire-bellied toad, the lowest average being over 50% *B. variegata* characters.

Regarding the first grid, the majority of the characters (1, 4, 5, 7, 8, 9 and 10) are expressed as *B. variegata* ones, registering values that vary between 68-100%. A very interesting situation can be observed within this grid, where major differences occur between the features of the two species. Thus, the second (the spots around the chin-chest area), third (the ones from the chest area) and the sixth character (the spots from the chest-abdomen area) are expressed in a proportion of 97%, 90% respectively 75% as *B. bombina* ones. The same situation can be observed in the case of the population near Oradea. This important difference is explained by STUGREN & VANCEA (1968) through the mutation phenomenon, thus the different features of the two species are sensed regarding the intensity selection, determined by the choice of the most advantageous characters.

The second grid also analyses 10 aspects. In this case there were 4 individuals that presented 100% *B. variegata* characters. The majority of the population comprises individuals that have *B. variegata* - like characteristics. Certain aspects were present at all of the individuals in a percentage of 100%, such as the first (it refers to the ventral colouring, which is yellow at *B. variegata* and red at *B. bombina*), second (the colour of the upper part of the first fingers and the top of fingers, which is yellow at *B. variegata* and black at *B. bombina*), third (the dorsal colouring is black at *B. bombina* and pale grey at *B. variegata*), sixth (the relation between the length and width of the head) and ninth character (the dorsal verrucae: sharp at *B. variegata* and smoothed at *B. bombina*). The 4th (the light spots from the tarsian and planter, which are united at *B. variegata* and separated through black pigment at *B. bombina*), 5th (the ratio between the dark pigment and the light one at the ventral side) and 10th character (the ratio of tibia-tarsian joints when the stylopode and zeugopode are parallel) are expressed as *B. variegata* ones, registering values between 75-90%. While character 7 (the presence at *B. bombina* of small white spots around the lateral-ventral verrucae and their absence at *B. variegata*) is a strong feature of *B. bombina* species, being present at 74% of the population. This situation can also be noticed at the *B. variegata*-like population from Oradea. Character 8 (the drawing of the dorsal part) is present at 48% half the population, thus at half of the individuals it is expressed as a *B. bombina* feature (regulated black tubercles) and at the other half as a *B. variegata* aspect (black scattered verrucae). Thus, the reduced weight of the 8th character is probably compensated by the exclusive presence of the sharp verrucae from the dorsal part of the individuals (character 9). Thus, as an adaptive feature it can be noticed the presence of the spines which secrete the highest quantities of toxic substances. At the same time, a refuge for the toad is presented through the grey silt, therefore a stained back with tubercles could represent a disadvantage for the population.

CONCLUSIONS

The trophic spectrum highlighted an overwhelming presence of the aquatic preys due to the rich content in larvae forms. Thus, because of the environmental conditions, the toads found plenty of trophic resources in the habitat, and therefore they did not have to leave the aquatic medium. However, the high weight of stomachs with content also suggests the existence of favourable conditions for the unfolding of the feeding activities. Secondly, the two grids emphasized a typical *Bombina variegata* - like population. Moreover, characters specific to this species could have been noticed, aspects which are determined by the relief and habitat's morphology.

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Dimancea Nicoleta, Covaciu-Marcov Severus-Daniel

University of Oradea

Str. Universității, No. 1, 410087, Oradea, Romania

E-mail: nicoleta_dimancea@yahoo.com

E-mail: scovaciu@uoradea.ro

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