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About a closed hybrid population between *Bombina bombina* and *Bombina variegata* from Oradea (Bihor county, Romania)

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Abstract. In 1997 there was recorded a perfect hybrid population nearby the Oradea Railway Station, which was mentioned in a population ranking study in 2001 (but only 21 samples were studied - Covaciu-Marcov et al. 2001). Our study had the following objectives: to analyse the affiliation of the population, analysing a larger number of individuals then in 2001; to estimate the population size; to analyse the food resource utilization of this population. The study period was April-July 2002. The affiliation study was relaying on the morphological and chromatic characteristics of the two parental species, using two gratings. The studied population is a typical hybrid population, despite the low altitude they were found at (140 m). The individuals are pretty much similar according to both gratings, respective according to their average. The Jolly-Seber estimate gives a population size of 515 toads. The small number of recaptured animals is surprising, because that this is a limited and closed population where no individuals leaved out or came in. Almost all studied individuals had stomach content. In the stomach contents we found vegetal remains, shed-skins and animal preys as well. The most important category of stomach contents was the animal prey's. The consumed animals were 32 categories. The essential food was Nematocera larvas, Coleoperas, Dipteras, Hymenopteras, Brahiceras, Homoptera Afidas and Crustacea Izopadas. There is an important variation for these groups of prey animals depending on the time of the year. In summer months the food diversity decreases compared to spring months. As the study succeeds the food similarity increases. In spring there are predominant terrestrial preys whilst in summer time the toads we've analysed eat in aquatic environment. We noticed that, the minimum number of samples necessary for feeding spectrum study varies depending on the time of the year. The taxonomical composition of preys, being a tight connection between the food availability and it's presence in the stomach contents. The type of feeding strategy (active foraging / sit-and-wait) used by the analysed toads, varies with the period of the study, abundance and accessibility of certain preys in the habitat. The high adaptability of their feeding strategies and the consuming of the most abundant prey shows a high ecological plasticity and an opportunist feeding behavior.

Key-words: *Bombina, hybrids, population size, feeding ecology*

Introduction

The two European species, from *Bombina* genus, share an important particularity the one of not being reproductive isolated, thus we can find a massive hybridization, in the contact areas between the two parental species populations (Szymura 1993). The length of the hybridization area is enormous, due to their habitat morphology in the Central Europe's relief (along the main mountain chains in this area). The hybridization area of *Bombina*

bombina and *Bombina variegata* species is known as one of the best examples of genetic interaction at the boundary of the species (Gollmann et al. 1993). The hybrids between the two European *Bombina* species first to be recorded were in Transilvania by Méhely (1905). These hybrids were obtained in the laboratory as well (Heron-Royer 1891), and there have also been seen pairs with each of the two species in amplex in the wild (Michalowshi 1958). Also in Romania the presence of these hybrids was known for a long time (Fuhn 1960, Cogălniceanu et al. 2000a).

From a biogeographycal point of view, the two species are vicariant species (substitute each other) (Bănărescu & Boşcaiu 1973), their habitats do not overlap each other but they come in touch. Despite the fact that their habitats may come in touch in a limited area, and here you can find both species, they still can be found in different habitats (Fuhn 1960, Covaciu-Marcov et al. 2003a), thus there is a mutual ecological vicariation (Bobrinschi 1953).

As many studies in this mater were accomplished in Europe (Michalowski 1958, Szymura 1976, 1988, Szymura & Barton 1991, Gollmann 1984, 1987, Gollmann et al. 1988, Köhler, 2003, Vines et al. 2003), there was possible to gather a lot of information regarding the hybrids, offering in the same time a unitary method on analysing them. In a similar way studies were done in Romania. More precise, there was studied the two European species of the *Bombina* genus area of hybridisation (Stugren & Vancea 1968, Stugren 1980, Ghira & Mara 2000, Ghira et al. 2003, Covaciu-Marcov et al. 2001, 2002a, 2003 a, 2004 a, b, Vesea et al. 2004). There have also been observed feature conservation for the two vicariant species: for *Bombina bombina* (Covaciu-Marcov et al. 2002 b) and as well for *Bombina variegata* (Covaciu-Marcov et al. 2003b).

In 1997 there was recorded a perfect hybrid population nearby the Oradea Railway Station (Covaciu-Marcov et al. 2001, Covaciu-Marcov 2004), which was mentioned in a population ranking study in 2001 (only 21 samples were studied - Covaciu-Marcov et al. 2001).

Our study had the following objectives:

1. - to analyse the affiliation of the population, analysing a larger number of individuals then in 2001 (Covaciu-Marcov et al., 2001);
2. - to estimate the population size;
3. - to analyse the food resource utilization of this population.

Materials & Methods

Study Period

The study period was April-July 2002. Each specimen was captured by bare hand or using special meshes. Just as soon as the specimens were analysed, they were released back into their natural environment.

Study area

The studied habitat consists of some 1 km long channel at the altitude of 140 m, at the low limit of the Oradea Hills, behind the Oradea Railway Station (photo 1). The channel's situated parallel to the railway, its width is maximum 30 cm and the depth is up to 20 cm. It is a man made channel, with concrete on the sides designed for residual water sloop, which eventually flows into the city's main waste channel. Hence, the channel is continuously supplied with water but it doesn't have its own vegetation but a thick layer of sludge (10 to 20 cm), which serves as the only place to hide for the hybrid's. On both sides of the channel there are many weeds (ex. *Ambrosia sp*). The habitat is insulated, being no connections between it and other biotopes with *Bombina*. The water is highly polluted with residual remaining, wastes and animal cadavers. The habitat is populated by numerous *Tubifycids* and *Hyrudineas* (*Haemopis sp*). We do not found any other Amphibian species in this habitat.

Analyzing the affiliation of the individuals in this population

The affiliation study was relaying on the morphological and chromatic characteristics of the two parental species. The morphological features are usually accurately clues for a good ranking of the species (Szymura 1993). We used the main features described by several authors (Stugren 1980, Golmann et al 1993, Szymura & Barton 1991, Ghira & Mara 2003, Ghira et al 2003) as a diagnostic method. Once we have standard features we can compare the results obtained on the hybridization area by different authors in different regions.

We have analysed 20 features using two gratings each with 10 features. Both of the gratings use a binary system (0,1). For each grating there will be a mark, 1 if the feature is a *Bombina variegata* one and 0 if it is a *Bombina bombina* one. Thus each individual can gather from 0 to 10 points (a ten points will be a pure *Bombina variegata* and a 0 points one will be a pure *Bombina bombina*). The individuals with a number of points close to 10 or 0 will be known as a *Bombina variegata*-like (7-8 points) and *Bombina bombina*-like individuals (2-3 points). The intermediate points: 4, 5, 6 represent the hybrids. The amount of features can be translated into percentage and a statistic can be made. Therefore an analysed population can be affiliated to one or another of the two species or ranked as a hybrid population.

The first grating analyses the morphology, dimension and ratios of the light ventral spots. These are red to *Bombina bombina* and yellow to *Bombina variegata.* For the first one, prevails the black pigmy and at the second one prevails the yellow pigmy (Fuhn 1960). There is considered the relation of ten groups of ventral light spots (tab. 1). The classification system was made by Szymura and Gollmann and also other foreign authors (Szymura & Barton 1991, Gollmann 1984, Gollmann et al 1993). If the light spots are separated by dark pigmy than the feature is for *Bombina bombina*; if the spots are joined together than it is a *Bombina variegata* feature.

Table 1. Grid 1 of differentiation of the European species of the *Bombina* gender (the characteristics of the ventral pattern) (Szymura & Barton 1991, Gollmann 1984)

Characteristic (light spots on):		***Bombina bombina***	***Bombina variegata***
1.	Chin – chin	*Separated*	*United*
2.	Chin – chest	*Separated*	*United*
3.	Chest – chest	*Separated*	*United*
4.	Chest – shoulder	*Separated*	*United*
5.	Shoulder – arm	*Separated*	*United*
6.	Chest - abdomen	*Separated*	*United*
7.	Abdomen – abdomen	*Separated*	*United*
8.	Abdomen – basin	*Separated*	*United*
9.	Basin – basin	*Separated*	*United*
10.	Basin – thigh	*Separated*	*United*

The second grating analyses ten features, as well, and it's been made by Stugren (1980) and modified by Ghira and Mara (2000) (tab. 2).

Table no 2. Grid 2 of differentiation of the European species of the *Bombina* gender (after Stugren 1980, modified by Ghira & Mara 2000)

	Character	*Bombina bombina*	*Bombina variegata*
1.	Colour of open ventral spots	*Red, orange, yellowish*	*Yellow*
2.	Colour of upper part of the first finger and the top of fingers	*Black*	*Yellow*
3.	Dorsal colouring	*Black*	*Pale grey*
4.	The relation tarsian and plantar open spots	*Separated*	*United*
5.	Ventral colour	*Orange spots on black background*	*Black spots on yellow background*
6.	The relation between the length and width of the head	*Length > width*	*Length < width*
7.	The drawing of lateral and ventral parts	*White spots around the verrucae*	*Without white spots around the verrucae*
8.	The drawing of the dorsal part	*Regulated black tubercles*	*Black scattered verrucae*
9.	Dorsal verrucae	*Lens – shaped, squatted*	*Sharp, rough*
10.	The ratio of tibia – tarsian joints when the stylopode and the zeugopode are parallel	*Not touching*	*Touching*

Due to the fact that the features are expressed in binary system in both gratings, we were able to analyse the similarities using the Sorensen similarity index (Chao et al. 2005) using EstimateS 7.0 software (Colwell 2005).

Estimating the size of the studied population

Identifying individuals is essential for population size determination (Krebs 1989). Therefore there is a wide range of marking methods for Amphibians (Ferner 1979). Because it is easy to use, the most often used method is the toe clipping method because frog's toes regenerate easy (Donelly et al. 1994, Halliday 1994). This method's disadvantage is the fact that it affects the behaviour, survival and the capture rate (Clarke 1972, Golay & Durrer 1994, Reaser 1995). For amphibians with characteristic pattern, the marking can be made using the chromatic differences (Cogălniceanu 1997). Pattern mapping (i.e., the identification of individuals based on natural, existing variation in colour patterns and other markings) is the less invasive marking method (Hangström 1973, Andreone 1986, Donnelly et al. 1994, Doody 1995). Thus, when estimating the population size for *Bombina bombina*, there is often used the belly patterns features (Brigs & Damm 2004).

When identifying the captured / recaptured individuals, we used not only the belly patterns but there were used the features from the two sets of gratings.

When estimating the population size we used the Jolly-Seber method (Ricker 1975, Cogălniceanu 1997). To apply this method you need repeated capture events with differentiated marking for each capturing (adequate for each capturing).

The number of individuals marked in the population at a time t_i (M_i) was determined with the following formula:

$$M_i = r_i + \frac{z_i m_i}{y_i}$$

M_i represents the number of animals captured, marked ands released at time t_i; and r_i represents the number of animals captured and marked at the time t_i; y_i represents the number of animals captured and released at the time t_i and than recaptured; z_i represents the number of animals captured and marked before the time t_i but no recaptured.

Knowing M_i's value we were able to estimate the population size using the following formula:

$$N_i = \frac{M_i(n_i + 1)}{(r_i + 1)}$$

The standard population size error is calculated as it follows:

$$ES_{N_i} = \sqrt{N_i(N_i - n_i)\left[\frac{M_i - r_i + m_i}{M_i}\left(\frac{1}{y_i} - \frac{1}{m_i}\right) + \frac{1}{r_i} - \frac{1}{n_i}\right]}$$

The feeding spectrum analyse

The 222 samples were collected monthly (between April and August 2002), which war necessary in order to monitor the calendar evolution of their feeding spectrum. We tried to capture a large number of frogs each month for samples collection.

We established the taxa affiliation for the prey animals, the frequency they've been consumed and the feeding intensity. The prey determination was done by order level and sometimes by family level, when it was possible. We didn't focussed our attention on a detailed classification of the prey taxa, but on their importance in the environment, therefore to have a better understanding on the *Amphibians* feeding (Meschersky 1997).

The method we used to obtain the stomach contents was the stomach flushing method (Griffiths 1986, Opatriňy 1980, Fraser 1976, Legler & Sullivan 1979, Leclerc & Curtois 1993). As soon as the stomach contents were collected the individuals were released in the provenience biotope, reducing in this way as much as possible our activity impact on the *Amphibians*.

The stomach contents were placed in airtight test tubes and they were preserved with a 4 % solution of formalin. The material was analysed in the laboratory with the aid of the microscope and the binocular magnifier. The preys were determined using the speciality literature (Radu & Radu 1967, Crişan & Mureşan 1999, Ionescu & Lăcătuşu 1971, Móczár 1990, Móczár et al. 1950).

Thus we were able to monitor the amount and frequency the prey taxa were consumed along the seasons. The amount represents the percentage of a certain prey being consumed out the total consumed preys. The frequency of occurrence was determined by dividing the number of stomachs that contained a particular prey by the total number of stomachs with prey.

The rate of feeding activity was estimated as the percentage of stomachs containing food with respect to the total number of stomachs examined (Sala & Ballesteros 1997).

The diversity of the diet was estimated with Shannon-Weaver (1949) diversity index (H) for each month of the study:

$$H = \sum \frac{n_i}{N} ln \frac{n_i}{N}$$

In the formula above, n_i is the number of prey category i and N is the total number of preys.

Rarefaction analysis was done for diet diversity of each month of study, based on suspects of m samples (where m varies between 1 and N frogs) using EstimateS 7.0 software (Colwell 2005). 1000 subsets were randomly chosen and an average measure was computed. The diversity accumulation curves obtained were used to determine the minimum sample size for each month. We accepted the sample size which gave at least 95% of the diversity calculated for the total sample size in each month.

We monitored the food similarity (niche overlap) among individuals and between two samples collecting periods.

The niche overlap between the months of study was estimated using Pianka's index (Pianka 1973):

$$Q = \frac{\sum p_{ij} p_{ik}}{\sqrt{\sum p_{ij}^2 p_{ik}^2}}$$

In the formula above p_{ij} and p_{ik} represents the abundance of prey category i in the food of predators j and k. The value of Q varies between 0 (no common resource) and 1 (perfect overlap). We used EcoSim 7.0 software (Gotelli & Entsminger 2001.).

The niche overlap among the individuals in the studied population was established using the Sorensen index (Chao et al. 2005), using the software EstimateS 7.0 (Chao et al. 2005).

Results

The affiliation of the studied population

The affiliation of 208 individuals was studied and they were captured in three different periods, respective tree different months (May-July). The recaptured individuals (were analysed once again) but they were not took into accountancy, therefore there was studied a total number of 164 individuals of *Bombina* genus.

Table 3. The affiliation of the studied population and the individual's similarities (S_{is}) according to the two gratings (G_1, G_2) and according to their average ($G_{1,2}$); SD is the standard deviation.

	G_1	G_2	$G_{1,2}$
%	49.75	45.72	47.61
S_{is}	0.72	0.66	0.7
SD	0.16	0.19	0.12

The population affiliation was established using the two mentioned gratings and finally we calculated the average between the two feature gratings. Considering the two gratings and the average between them both showed that the population from Oradea Railway Station was a perfect hybrid population (tab. 3). Both gratings prove that the majority of the individuals have the features of the two species in equal percentages (tab. 4). The mutual similarities of the analysed samples are rather high according to both feature gratings, and to calculated average between the two gratings (tab. 3).

Table 4. *The features amount* (C_{1-10}), according to the two gratings (G_1, G_2), for the studied populations.

		C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
G_1	*%*	87.98	7.93	6.25	23.55	90.14	12.25	80.28	50.72	68.5	69.95
	SD	0.32	0.25	0.24	0.4	0.23	0.32	0.39	0.47	0.46	0.38
G_2	*%*	87.01	76.44	16.34	28.12	90.38	11.53	56.49	29.56	54.8	6.49
	SD	0.33	0.34	0.37	0.41	0.28	0.32	0.49	0.45	0.48	0.24

The majority of studied individuals were hybrids (73.17 %) (tab. 5). A low percentage of *Bombina bombina* was found (1.22 %). All the other individuals had features that made them more like one of the other two parental species. The *Bombina bombina*-like individuals were 17.07 % compared to *Bombina variegate*-like ones in a percentage of 8.53 %. We do not found any samples with *Bombina variegata* phenotype.

Table 5. The ratio of morphology-types at the studied population:

	Bb*	***Bb*-l***	**H***	***Bv*-l***	*Total*
no.	2	28	120	14	164
%	1.22	17.07	73.17	8.537	100

**Bb - B. bombina; Bb*-l - *B. bombina;* H - hybrids; *B.v.*-l - *B. variegata*-like

The population size estimation

Between May and July 2002, doing three repeated catching actions, we captured a total of 208 hybrid individuals. Among these there were 44 recaptured ones. We estimated the population size using the Jolly-Seber method (Cogălniceanu 1997). The Jolly-Seber estimate gives a population size of 515 toads with a standard error of 184 (tab. 6). The standard error represents 35.8 % of the total estimated number.

Table 6. The estimated size of the population (N_i); *SE* – standard error.

M_i	N_i	*SE*
90.18	***514.6***	184.2

The results of the feeding spectrum study

In this respect there were analysed 222 individuals (tab. 7). Almost all studied individuals had stomach content. Toads with no stomach content were found only in April, July and August. The contents had, besides animal type, vegetal type and shed skin of other individual's, in the population as well. There were found vegetal rests appear in high amount in each month of the study.

The most important category of stomach contents was the animal prey's one. The consumed animals were 32 categories of invertebrate (tab. 9). We separated the larvae and adults for Coleoptera's, Diptera's and Nematocera's, because we considered, they represent different categories, as far as mobility and provenience environment are concerned. Redford & Dorea (1984) and Bell (1990) states that the adult of Insects do not differ in nourishing content is concerned. But still it is reckoned that the larvae and pupae of the holomethabole Insects, are richer in fats and hence more nourishing than adults (Brooks et al. 1996).

Table 7. The number of analysed individuals; the number of stomachs with contents; the number of stomachs with vegetal rests and shed skin; SD-standard deviation:

	April	*May*	*June*	*July*	*August*	*Total*	*SD*
No. of samples	21	60	52	50	39	222	-
%Stom with contents	93.34	100	100	94	97.44	96.4	3.18
%Stom with vegetable	76.19	71.66	76.92	60	87.17	73.42	9.84
%Stom with shed-skin	4.76	13.33	15.38	20	12.82	14.41	5.53
% Stom with minerals	0	3.33	1.92	0	0	1.35	1.52

When estimating the feeding intensity we used the maximum and medium number of preys/individual (tab. 8). The highest value for the medium number of preys was recorded in June (15) while for the maximum number of preys the highest value is in summers month.

Table 8. The number of prey items, the average and maxim number of prey items / samples

	April	*May*	*Iuny*	*July*	*August*	*Total*	*SD*
No. of prey items	131	379	801	521	422	2254	
Average no. of preys	6.23	6.3	15	10.4	10.8	10.15	3.65
Maxim no. of preys	19	38	65	40	83	83	25.6

In April the analysed toads consumed a relative equal number of preys with low, medium and high mobility (table10). In summer time there was noticed a decrease in the number of high and medium mobility preys; whilst the low mobility ones are more frequent consumed. In parallel, there was noticed a transition from eating lonely life preys to eating animals that live in groups. Thus, in April and May, group life preys are reduced, whilst in summer months they are most frequently consumed.

Food is more divers in the stomach contents in the first month of the study (2.13) (tab. 11). In summer months the food diversity decreases (compared to spring months), to values close to 1. Feeding similarity among the analysed individuals was quite high in almost every month of the study (Tab.11). As the study succeeds the food similarity increases too (tab. 11, 12).

Photo 1 The studied habitat nearby the Oradea Railway Station

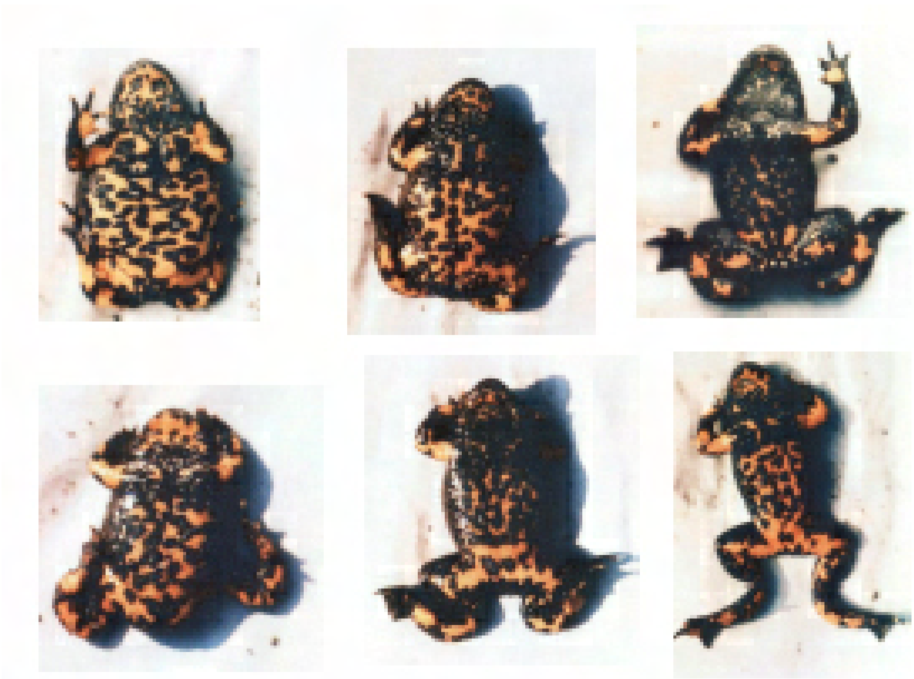

Photo 2 The belly pattern of some analysed samples

The essential food was Nematocera larvas, Coleoperas, Dipteras, Hymenopteras, Brahiceras, Homoptera Afidas and Crustacea Izopadas (tab. 9). There is an importance variation for these groups of prey animals depending on the time of the year. The amount of Homoptera Afida's, Crustacea Izopoda's, Hymenoptera Formicida's, Coleoptera's and the Brahicera adults are important only in spring; they are less important in summer. Unlike the above mentioned, the Diptera Nematocera larva's are becoming more and more important towards summer. The frequency is correlated with the amount of preys. A particular situation is the spiders, which are very frequent but their amount do not represent an essential value for the Amphibians feeding.

Table 9. The prey amount (A%) and the frequency of preys (F%):

	A (%)							*F(%)*						
	April	*May*	*Iuny*	*Iuly*	*August*	*Total*	*SD*	*April*	*May*	*Iuny*	*Iuly*	*August*	*Total*	*SD*
Oligocheta - Lumbricida	-	-	-	0.57	0.94	0.31	0.43	-	-	-	4	7.69	2.25	3.45
Gasteropoda - snails	-	0.52	0.12	0.38	1.42	0.48	0.55	-	3.33	1.92	4	10.25	4.05	3.86
Gasteropoda - Limax	0.76	0.26	-	-	0.94	0.26	0.43	4.76	1.66	-	-	2.56	1.35	1.99
Araneid	3.81	0.52	1.62	1.15	1.42	1.41	1.24	23.81	3.33	23.07	8	15.38	13.06	9.05
Acaria	2.29	0.26	0.12	-	-	0.22	0.98	14.28	1.66	1.92	-	-	2.25	6.05
Crustacea - Izopoda	14.5	5.54	1.49	-	1.89	2.66	5.85	47.61	8.33	13.46	-	12.82	12.16	18.23
Miriapoda-Diplopoda	4.58	0.26	-	-	0.23	0.35	1.99	23.81	1.66	-	-	2.56	3.15	10.23
Colembola	2.29	0.52	-	-	0.23	0.26	0.96	4.769	3.33	-	-	2.56	1.8	2.09
Odonata - larva	-	-	-	-	0.47	0.08	0.21	-	-	-	-	2.56	0.45	1.14
Ortoptera - larva	-	-	0.24	-	0	0.08	0.11	-	-	3.84	-	-	0.9	1.72
Heteroptera - imago	0.76	0.52	1.12	0.95	0.47	0.84	0.27	4.76	3.33	13.46	8	5.12	7.2	4.02
Homoptera - Afida	8.39	12.66	0.87	0.76	-	3.1	5.68	9.52	30	11.53	2	-	12.16	11.88
Homoptera - Cidadina	-	0.79	0.62	0.38	0.23	0.48	0.31	-	5	5.76	4	2.56	4.05	2.27
Coleoptera - larva - undet. T	-	0.26	0.24	0.19	1.89	0.53	0.77	-	1.66	1.92	2	12.82	3.6	5.17
Coleoptera - larva - *Dytiscida*	-	0.52	0.99	-	-	0.44	0.44	-	3.33	11.53	-	-	3.6	5
Coleoptera - undet.	3.81	13.45	2.37	1.53	0.94	3.85	5.16	19.04	38.33	28.84	16	10.25	24.32	11.12
Coleoptera - Dytiscidae	-	-	-	-	0.23	0.04	0.1	-	-	-	-	2.56	0.45	1.14
Coleoptera - Coccinelida	-	1.31	2.12	0.76	-	1.15	0.9	-	6.66	26.92	8	-	9.9	11.03
Coleoptera - Elaterida	0.76	-	-	-	-	0.04	0.34	4.76	-	-	-	-	0.45	2.12
Coleoptera - Scarabida	-	-	-	0.19	-	0.04	0.08	-	-	-	2	-	0.45	0.89
Coleoptera - Carabida	-	0.26	-	-	0.23	0.08	0.13	-	1.66	-	-	2.56	0.9	1.2
Coleoptera - Curculionida	-	0.79	0.12	-	-	0.17	0.34	-	5	1.92	-	-	1.8	2.18
Coleoptera - Stafilinida	-	0.52	0.37	0.38	-	0.31	0.24	-	3.33	5.76	4	-	3.15	2.55
Lepidoptera - larva	-	-	0.12	0.19	0.71	0.22	0.29	-	-	1.92	2	7.69	2.25	3.15
Diptera - Nematocera - larva	3.05	37.46	81.64	83.3	80.09	69.74	35.79	14.28	38.33	80.76	82	69.23	61.26	29.63
Diptera - Brahicera - larva	9.92	0.52	0.74	0.19	-	0.97	4.28	33.33	3.33	5.76	2	-	5.85	13.82
Diptera - Brahicera - pupae	-	-	-	0.76	-	0.17	0.34	-	-	-	4	-	0.9	1.78
Diptera - Nematocera - imago	3.05	0.26	0.12	0.57	1.65	0.7	1.22	14.28	1.66	1.92	6	12.82	5.85	5.94
Diptera - Brahicera - imago	31.29	1.31	1.24	1.72	1.89	3.23	13.3	71.42	8.33	15.38	12	15.38	18.01	26.39
Hymenoptera - undet	0.76	2.37	0.49	2.87	-	1.28	1.25	4.76	10	7.69	28	0	11.26	10.68
Hymenoptera - Formicida	9.92	18.73	3.12	3.07	4.02	6.29	6.75	42.85	45	34.61	24	23.07	33.78	10.23
Hymenoptera - Apida	-	0.26	-	-	-	0.04	0.11	-	1.66	-	-	-	0.45	0.74

Another aspect of our study was the determination of the provenience environment for the prey (tab. 10). The prevalent amount was the aquatic one (71.13 %). There is, though, a seasonal variation for preys with different provenience. Therefore, in spring there are predominant terrestrial preys whilst in summer time the toads we've analysed eat in aquatic environment.

Table 10. *The amount of prey taxa depending on their mobility and provenience:*

	April	*May*	*Iuny*	*Iuly*	*August*	*Total*	*SD*
Terrestrial	87.21	61.67	16.47	16.85	19.19	28.86	32.48
Aquatic	12.78	38.32	83.52	83.14	80.8	71.13	32.48
Low	31.29	54.08	85.51	86.75	87.2	77.68	25.31
Medium	23.66	22.95	9.23	4.99	5.21	10.64	9.37
High	45.03	22.95	5.24	8.25	7.58	11.66	16.74

Table 11. Food diversity (H_{lim}), minimum number of samples (n_s), food similarity among individuals (S_{is}); *SD*-standard deviation:

	April	*May*	*Iuny*	*Iuly*	*August*	*Total*	*SD*
S_{is}	0.31	0.19	0.36	0.36	0.26	0.24	0.07
SD_s (S_{is})	0.22	0.26	0.24	0.27	0.27	0.26	/
H	2.13	1.9	0.92	0.85	1	1.42	0.6
H_{lim}	2.02	1.8	0.91	0.79	0.94	1.34	0.57
SD_s (H)	0.27	0.19	0.03	0.06	0.09	0.07	/
n_s	10	21	4	13	5	14	4.97

Table 12. Food similarity along the study (according the Pianka's indexes)

	May	*Iuny*	*Iuly*	*August*
April	0.32	0.11	0.11	0.12
May		0.83	0.83	0.83
Iuny			0.99	0.99
Iuly				0.99

Using rarefaction curves we could establish the minimum number of samples necessary for each month (tab. 11). We considered a sufficient number of samples containing 95% (H_{lim}) of the maximum variety of prey (H) (Kovács Török, 1997). We noticed that, depending on the time of the year, the minimum number of samples varies from 2 (in June) to 21 (in May).

Discussions

The affiliation of the studied population

The population from Oradea Railway Station is a typical hybrid population, despite the low altitude they were found at (140 m) (photo 2). It is remarkable that in the study area the hybridization line comes unusually low (between 150 and 250 m); whilst in

Transilvanian Plateau, for instance, the hybridization line is on 400m (Covaciu-Marcov et al. 2000). In the studied area, *Bombina variegata* gets down to 150 m, whilst *Bombina bombina* is found even at 250 m (Covaciu-Marcov 2004).

The occurrence of a number of *Bombina bombina* phenotype, may be a consequence of the studied environment's altitude. In a previous study made only on 21 individuals there were found none *Bombina bombina* individuals. Most probably, due to the low altitude there is a more frequent occurrence for the *Bombina bombina-like* individuals compared to *Bombina variegata-like* individuals.

The individuals are pretty much similar according to both gratings, respective according to their average. Here, there are mere differences between the most different individuals compared to other studied hybrid populations from the county of Bihor (Covaciu-Marcov et al. 2001, 2002 a, 2003 a, 2004 a, b, Vesea et al. 2004). This shows there is a balanced and autonomous hybrid population, without parental phenotype adidion. An important fact is that this population is insulated; there are no other *Bombina* populations on a range of a few miles all the way around (Covaciu-Marcov 2004). Most probably, the individuals with *Bombina bombina* phenotype, are the result of hybrid mating not other pure species.

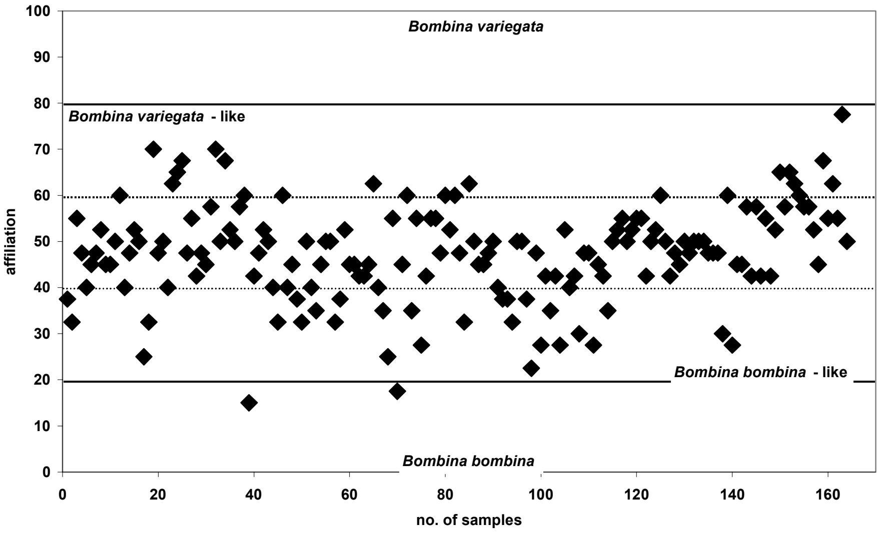

Diagram 1. The affiliation of individuals in the studied environment

Compared to other studied populations around Oradea city there are significant differences according to the two gratings, at the Oradea Railway Station the hybrid individual's features are close to the centre of the grating (Covaciu-Marcov et al. 2001). Probably the hybrid population evolves independently for a while and leads to stable intermediary phenotypes, which lasts in time (Covaciu-Marcov 2004). As log as there is contact with individuals from outside the population there is maintained a high polymorphism (Covaciu-Marcov et al. 2001). Two genetic studies on amphibians have found steep increases in genetic differentiation with increased in inter pond distance (Reh &

Seitz 1990, Hitchings & Beebee 1997). Both studies found that urban development is positively correlated with genetic divergence amount populations. Open populations have a highly variable recruitment, while closed populations should have less variable reproduction. In closed populations, more adults ultimately beget more adults, whereas there is no guarantee of this for open populations (Halpern et al. 2005).

If we compare the results of our previous study made on this population (Covaciu-Marcov et al. 2001), we can see that this population maintains itself constant, their characteristics being similar in each tear of study. Thus, this hybrid population has a theoretical and practical value for the studuies of hybrids between *Bombina bombina* and *Bomnia variegata*.

The presence of the population was known since 1997 (Covaciu-Marcov et al. 2001, Covaciu-Marcov 2004) and it survives in a highly polluted environment, which is probably a special adaptation of hybrids to extreme environment conditions. In a similar way, along the *Bombina's* hybridisation zone in Bihor County, where the habitats are intensely influenced by human activity, most of the individuals are hybrids; only a small number of parental species are found among these hybrid populations, despite the presence in the immediate neighbourhood (50 m distance) of parental species (Covaciu-Marcov et al. 2003 a). The structure and the altitude of the habitats is, probably, the cause for the morphotypes selection, as in many other examples of *Bombina* populations in Bihor County (Covaciu-Marcov et al. 2003).

Estimating the size of the population

We considered the individual marking method, the most efficient one in order to estimate the population size. For the European *Bombina* species there were realised genetic estimations and (*Bombina bombina*) (Szymura & Barton 1991) and male vocal based estimations (Briggs & Damm 2004). In the case of male based estimations, can have significant errors due to the fact that the sex ratio of males to females in breeding ponds is always male biased (Petranka 1998). This fact is caused probable the lower hibernation survival rate for them, because there were previously used the reserve substances for laying eggs. Similar events were stated la another Amphibian species before (Holenweg & Reyer 2000, Loumbourdis & Kyriakopoulou-Sklavounou 1996). Hence estimations based only on males counting are relatives and there were needed more accurate estimate methods (Stumpel 2004).

A primary purpose in ecology is to identify the processes and the factors that limit the local populations size of species (Halpern et al. 2005). Extreme conditions, where a habitat required by a particular life stage is completely absent or in excessive abundance (in the case of *Bombina* genus populations), set clear boundary limits for how habitat availability affects population size (complete limitation vs. no limitation, respectively) (Halpern et al. 2005). For closed, limited populations the populatin size is dictated by the size of the habitat.

The small number of recaptured animals is surprising, knowing that this is a limited and closed population where no individuals leaved out or came in. One explanation would be for them being eaten by predators. It is not a good explanation because these toads are producing noxious mucus secretion (Fuhn 1960), massive reducing their predators number. Also because the habitat is very much in contact with human activity, the number of possible predators is reduced (to leech and some Insects larva). The only plausible explanation is that the population is a large one. The large number of individuals in this population is the key to its survival in an more often modified environment by human activity (mud removing – by the railway station workers). Which also stands to back up the previous affirmation. In many cases, the extinction of a local population (the studied population being considered a local population) is totally due to the destruction of the habitats (Sinsch 1992). It is well known that many Amphibian species are short lived and a few bad years will lead to population extinction. In the small bodied toads of the genus *Bombina*, longevity can reach 10-12 years in *Bombina bombina* (Ananjeva & Borkin 1979, Smirina 1994, Briggs 1996, Kinne et al. 2004) and 12 years in *Bombina variegata* (Sy & Grosse 1998 – estimated from succesive recaptures). For both parental species, though, there were recorded 20 years old individuals (*B. variegata* - Płytycz & Bigaj 1993, *B. bombina* – Mecke personal communication in lit.). Thus, the survival of this hybrid population could be due to their longevity too.

The hybrid population persist (being a success), is important because spatial processes such as habitat fragmentation and lose of dispersal corridors may be increasingly important causes of amphibian population declines (Blaustein et al. 1994, Hecknar & M'Closkey 1996, Beebee 1997). A number of studies have found that urbanization and roads may limit amphibian dispersal or abundance (Fahring et al. 1995, Lehtinen et al. 1999). Generally, many Amphibians (as the *Bombina* genus) are highly phylopatric organisms, with low spreading abilities (Blaustein et al. 1994, Duelman & Trueb 1994, Sinsch 1990). Thus, the ponds isolation is a determinant factor for the viability of some populations of Amphibians (Laan & Verboom 1990, Blaustein et al. 1994), becoming a key element for their survival along the time.

There are known manipulative experiences in the case of many Amphibian populations, when fragmentation can be simulated by including habitat isolation with artificial barriers (Murdoch et al. 1996). Thus, the studied hybrid population (formed by a large number of individuals) has a theoretical and practical importance, because it had for a long time separated (landscape effects) compared to local habitat quality effects on the studied population.

In Denmark were observed *Bombina bombina* adults, which are migrating in breeding season from a pond to another (Briggs & Damm 2004). Feeding ponds are usually full of vegetation, being an eutrophyc one. *Bombina bombina* frequent migrate from breeding ponds to foraging ponds and back again (200-300 m distance) (Briggs 1993). The hybrid population we've studied uses the same pond as breeding and foraging pond.

The feeding spectrum

The amphibians spend a long period of their life searching for food which is a key preoccupation in their ecology (Perry et al. 1990). There is already well known information related to the two parental specie's trophyc spectrum, for *Bombina bombina* (Medvedev 1974, Gocharenko et al. 1978, Tertyshnikov & Goroyava 1982, Sas et al. 2003 a, 2004 a, Lác 1958, 1959, Orságová 1969, Ratajskỳ & Vojtková 1971, Ščerbak & Ščerban 1980) and as well for *Bombina variegata* (Sârbu 1976, Sas et al. 2004 b, Nemes & Petráss 2003, Taraščuk 1959, Kminiak 1978, Ščerbak & Ščerban 1980, Kuzmin 1990, Orságová 1969), but not any related to their hybrid's.

The reduced number of empty stomachs indicates that there were optimal feeding conditions, even if the environment was man-modified. Usually the Amphibians consume a range of invertebrates. Vegetal fragment being swallowed accidentally, while swallowing the animal preys (mobile preys) (Whitaker et al. 1997), therefore we didn't find any exclusive vegetal stomach contents. A similar situation was encountered to *Bombina bombina* (Sas et al., 2003a, 2004a). and *Bombina variegata* (Sas et al., 2004b, Sas et al. in press) populations.

Some authors believe that the Amphibians shed-skin eating is a habit for epidermal protein recycling (Weldon et al. 1993), but we believe it's presence in Amphibians stomachs is accidentally when taking action at other individuals movements. They can't eat prey their own size, so all they get can be a fragment of shed skin. Shed skin eating is, though, a frequent phenomenon with Amphibians, for European species of *Bombina* genus (Sas et al. 2003a, 2004 a,b) and other species of *Anura* (Sas et al. 2003b) and *Urodela* as well (Gunzburger 1999).

Prey diversity changes a lot during the seasons. Even if in April, due to low food availability, there have occurred only a few prey animals in the stomach contents, the food diversity is higher for the analysed toads. In summer months the frogs consume many types of prey animals but still with a lower diversity. This is due to progressive abundance growth of Nematocera larvae in the habitat. These are small larvae and live in groups, therefore they are captured in a large number as they're found; the toads trying to capture as many as possible in order to supply their bodies with the necessary energy. In Hungary, Kovács and Török (1997) have analysed the food diversity for *Bombina bombina* with a result of 1.81. According to our results, the food diversity was similar or even higher compared to the one noticed by these authors, but only in spring months, for *Bombina* hybrids. Opposite, in summer months, due to the Nematocera larvae presence, the food is much less divers than the one observed to *Bombina bombina* (Kovács & Török 1997). On the other hand, for *Bombina variegata* there was recorded a food diversity of 3.79 (Sas et al. in press). This is due to the difference in the two parental species and the hybrids's habitat structure. *Bombina variegata* occupies aquatic habitats of small dimensions (Fuhn 1960) hence it is obliged to hunt in terrestrial environment (leaving not much access to preys that live in groups); food diversity becoming more divers.

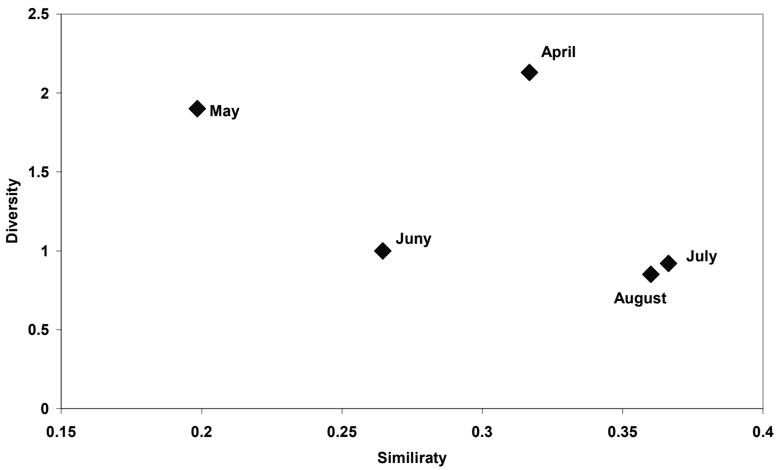

Diagram 2. Food diversity and similarity correlation in each months of study

The value for average of prey number / individual is increased compared to the parental species (*Bombina variegata* - Sas et al. 2004 a, in press; *Bombina bombina* – Kovács & Török 1997). As explained above, the difference was made by the Nematocera larvae which were easy to capture in a large number. Identically, there is an increasing feeding niche overlap (similarity) between two adjacent months caused by the high consuming of mosquitoes larvae. The high similarity food rate among individuals is explained by the use of the same food type which was available, especially Nematocera larvae. Close similarity was found at a *Bombina bombina* population in Hungary (0.30) (Kovács & Török, 1997).

The minimum number of samples for feeding spectrum analyses differs during the study period, hence it is important to mention the time for the sample gathering. The cause of this variation is in connection to increasing or decreasing of the prey abundency in the habitat. Thus, we believe it is important to state the period of sample gathering when made. The minimum number of samples for the entire period of the study is 14 to 15, similar with the one from the Hungarian study for *Bombina bombina* (13) (Kovács & Török 1997).

The Amphibians eat all types of animals with appropriate size in order to be swallowed (Török & Csörgő 1992). The taxonomical compoyition of preys, being a tight connection between the food availability and it's presence in the stomach contents, having thus an example of natural selection at feeding spectrum level.

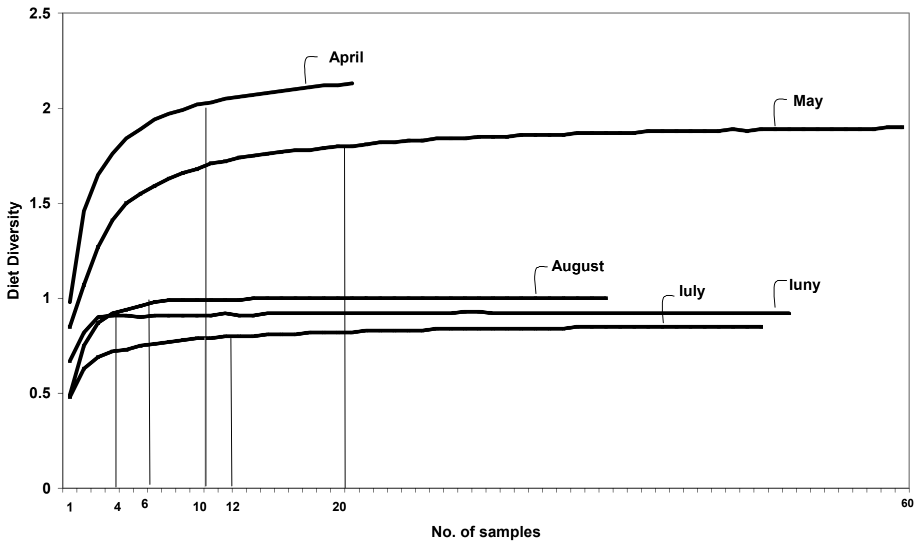

Diagram 3. The minimum number of preys in different periods

The hybrids (and the parental species too) are in a way confined to aquatic environment (Cogălniceanu et al. 2000b), hence it is expected for them to consume aquatic prey. But in the first two months (before the Nematocera larvae are abundant in the habitat) the terrestrial preys was more frequent consumed. It doesn't mean the fgrogs hunted in terrestrial environment, terrestrial animals floating on the water (spiders) and on the aquatic plants (flying insects). For other species of Anura were observed to have consumed a large number of terrestrial preys (Lőw et al., 1990, Sas et al. 2004c). Another observation was made on the two parental species: *Bombina variegata* usually eats terrestrial preys, when *Bombina bombina* usually hunts in aquatic habitat. In conclusion the differences for consumed preys for all, the parental and hybrid populations, are due to their prey availability (abundance). Thus, when the abundance of aquatic prey rises, their consuming rises too and the amount of aquatic preys as well.

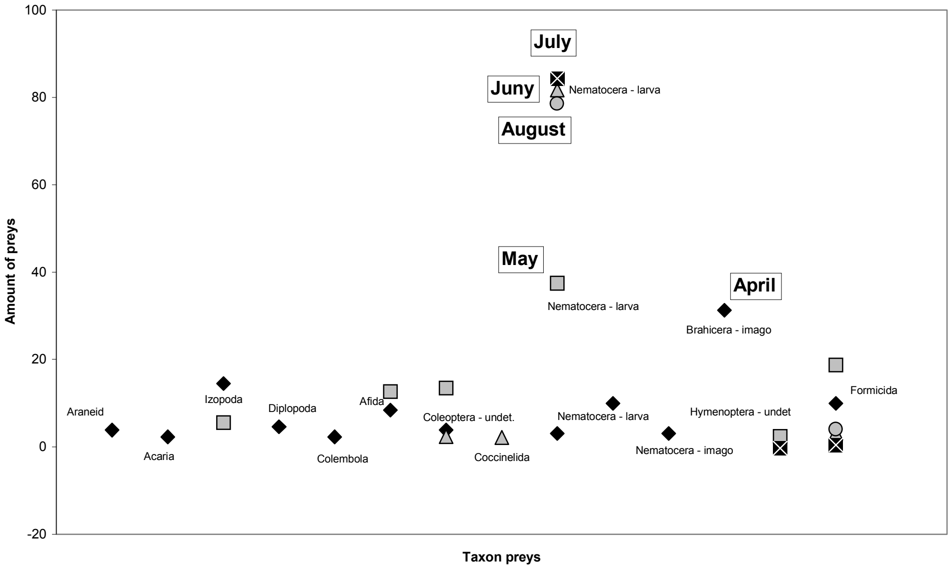

Diagram 4. Preferably prey categories during the periods of the study

The analysed frogs were using the active-foraging strategy (Huey & Pianka 1981) and the sit-and-wait feeding strategy too (Perry & Pianka 1997). The number of frogs using a certain feeding strategy varies with the period of the study, abundance and accessibility of certain preys in the habitat. Thus, it is observed a frequent consume of high mobility preys only in April. In the summer months, the number of high mobility prey consumed decreases and the number of preys with low mobility and group life increases.

The high adaptability of their feeding strategies and the consuming of the most abundant prey shows a high ecological plasticity and an opportunist feeding behaviour.

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