DATA UPON THE FEEDING OF TWO *EPIDALEA VIRIDIS* POPULATION FROM DOBROUDJA, ROMANIA

SÁRA FERENŢI, DIANA CUPŞA, NICOLETA DIMANCEA, BOGDAN HORIA VLAD, ANIELA FILIMON

Abstract. *We analysed the trophic spectrum of two Epidalea viridis populations from Dobroudja, around St. Ap. Andrei and Cocoş monasteries, during the period of 08-25 August 2007. The appearance of the different preys is influenced by the environmental conditions, the frogs feeding mostly terrestrial. The taxa with the highest weights were the Formicida, the formic acid from their body being used for the venom secretion of the frogs' skin. The prey diversity depends on the habitat, the maximum one being registered in the case of the Cocoş monastery.*

Keywords: *trophic spectrum, Dobroudja, Epidalea viridis.*

Rezumat. Date asupra hrănirii unei populaţii de *Epidalea viridis* din Dobrogea, România. *Am analizat spectrul trofic la două populaţii de Epidalea viridis din Dobrogea, din jurul manastirilor Sf. Ap. Andrei şi Cocoş în perioada 08-25 August 2007. Apariţia diferitelor prăzi este influenţat de condiţiile mediului, hrana broaştelor fiind majoritar de provenienţă terestră. Taxonul cu ponderea cea mai mare erau Formicidele, acidul formic din corpul lor fiind folosit pentru secretarea veninului din pielea broaştelor. Diversitatea prăzilor depinde de habitat, maximul fiind înregistrat în cazul mănăstirii Cocoş.*

Cuvinte cheie: *spectrul trofic, Dobrogea, Epidalea viridis.*

INTRODUCTION

It is known that amphibians can successfully inhabit dry environments, characterized by intermittent availability of water (SICILIA et al., 2006). The amphibians have diverse adaptations in order to successfully survive in the terrestrial medium, such as the modification of the skin permeability and the decrease of urine secretion (JORGENSEN, 1997). Moreover, other adaptations are the usage of temporary puddles only for reproduction and the rapid metamorphose of the tadpoles (HUSSEIN & DARWISH, 2000). Further research shows that the way in which the amphibians use the terrestrial habitats can be much more complex that is was previously thought to be (HOPKINS, 2007). This was the reason that many authors started to reassess the importance of the terrestrial habitats near the moist areas (SEMLITSCH & ROTHERMEL, 2003; GIBBONS, 2003).

Epidalea viridis is an eurithop species, it is considered to be a pioneer species, having the characteristic to live in regions with a dry and warm climate (PUKY et al., 2005), including on sandy substratum (ARNOLD, 2002a). The *Epidalea viridis* species is a xerothermophilous element, being considered one of the most common species from Dobroudja (COVACIU-MARCOV et al., 2006 a), while in the western and northern part of the country it is less commonly encountered, due to the colder and wetter climate (COVACIU-MARCOV et al., 2003 a, 2004 a). Its typical reproductive sites are temporary and shallow water bodies (BOLOGNA & GIACOMA, 2006). Studies about the trophic spectrum of this species have been published by many authors (COVACIU-MARCOV et al., 2005; HUSSEIN & DARWISH, 2000; NICOARĂ et al., 2005; DAVID et al., 2008).

The objective of our study is to analyse the trophic spectrum of two *Epidalea viridis* populations, highlighting the differences between them, respectively the adaptations of their trophic spectrum towards the terrestrial environment.

MATERIAL AND METHODS

We analysed two *Epidalea viridis* populations from Dobroudja, from Constanţa and Tulcea County during 8-25 August 2007. The investigated frogs from each population were collected around St. Ap. Andrei, respectively Cocoş monasteries.

St. Apostle Andrei monastery is surrounded on one side by an agricultural field, and on the other side by a forest, which is partly affected by the presence of the refuse. The humidity of the area is maintained by fountains, a canal that dries out in the droughty period, and by temporary puddles that are mainly situated inside the forest.

The habitat from Cocoş Monastery is slightly different from the previous one, being less anthropically affected. The forest is richer near the monastery, the reproducing habitats also being represented by the temporary puddles from the forest.

The collecting of the analysed samples was made directly by hand after nightfall, at the electric lights. The stomach samples were drawn with the help of the stomach flushing method, which is recommended by many authors (SOLÉ et al., 2005, SOLÉ & PELZ, 2007, CAPUTO & VOGT, 2008, CECALA et al., 2007). This is a non lethal technique for the analysed frogs. The stomach contents were preserved in formaldehyde and analysed using the scientific literature (RADU & RADU, 1967, STEINBACH et al., 2000; CHINERY, 1998; PAULIAN, 1971).

The results were statistically analysed, the followed parameters being the taxonomic affiliation of the preys, the feeding intensity, the rate of the feeding activity, the origin, weight and frequency of preys, the food diversity (the Shannon Wiever index) (SHANNON & WEAVER, 1949), food similarity (the Sorrensen index) (CHAO et al. 2005) and the differences that appear between the two habitats (the Mann Whitney test), using Estimates 7.0 software (COLWELL 2005). Thus, the obtained data were comparatively explained, analysing the differences that appear between the trophic spectrums of the two *Epidalea viridis* populations.

RESULTS

The *Epidalea viridis* population from St. Apostle Andrei Monastery was studied between the 8th and 9th of August 2007, while the one from Cocoş Monastery was investigated on the 25th of Agust 2007. We captured 19 individuals, respectively 12 samples of *P. viridis* from St. Apostle Andrei Monastery, while 56 individuals from Cocoş Monastery. Although, at St. Ap. Andrei Monastery, the difference between the drawing of the samples is of one day, relatively high differences appear, which suggest that in order to obtain a better picture of the trophic spectrum of this population, it is necessary to repeatedly perform stomach flushing, probably also due to the lower number of individuals.

In the case of St. Ap. Andrei Monastery, the consumed preys were grouped in 28 taxonomical categories, while regarding the other population they belong to 21 categories. The preys were classified only until an order or family level, eventually specifying their stage of development, respectively their origin.

Regarding the feeding intensity, we analysed several parameters. The number of consumed preys was much higher in the case of the population from St. Ap. Andrei Monastery, even if the number of studied individuals was much lower. The average number of preys/individual, similar to the previous data was very high, being over 40 on every date, while at Cocoş Monastery it was 6.51. The maximum number of preys is also very high at St. Ap. Andrei Monastery (311).

Concerning the feeding activity rate, at St. Ap. Andrei Monastery it is of 100 %, which corresponds with the very high feeding intensity. In the case of the population from Cocoş Monastery, 7 individuals did not present stomach contents, the weight of the empty stomachs registering a value of 3.36 %.

Generally, the preys are 100 % terrestrial, just in the case of St. Ap. Andrei Monastery, on the first day 0.25 % preys appear with an aquatic origin.

Table 1. The feeding intensity, the food diversity and similarity, the frequency of vegetal and shed skin fragments.
Tabelul 1. Intensitatea hrănirii, diversitatea şi similaritatea hranei, frecvența fragmentelor de vegetale şi exuvie.

Locality	**St. Ap. Andrei Monastery**			**Cocoş Mon.**
Date	**August 8**	**August 9**	**TOTAL**	**August 25**
No. of studied individuals	19	12	31	56
Total no. of preys	786	1.148	1.934	365
No. of empty stomachs	-	-	-	7
Maximum no. of preys/individual	115	311	311	28
Average no. of preys/ individual	41,36	95,66	62,38	6,51
Diversity	1.62	0.67	1.14	1.78
Similarity	0.47	0.54	0.49	0.23
Vegetal	84.21	41.66	67.74	48.2
Minerals	10.52	16.66	12.90	7.14
% Aquatic preys	0,25	-	-	-
% Terrestrial preys	99,75	100	100	100

Regarding the weight of the prey taxa, we can observe certain differences between the habitats, but also some variations in the trophic spectrum of the population from St. Ap. Andrei Monastery. Depending on the habitat, there are certain preys that appear only in the case of one habitat. The Hymenoptera Formicida held the maximum weight at St. Ap. Andrei Monastery, preys that also appear at Cocoş Monastery, but with a much lower weight. The Coleoptera Carabida registers a second place, followed by the Heteroptera. The weight of the Coleoptera is higher at Cocoş Monastery. In addition, in the case of this habitat, the Araneida appear with a relatively high weight, followed by the terrestrial Heteroptera, respectively the Brahicera adults. The weight variation of the Coleoptera from day to day is interesting at the population from St. Ap. Andrei Monastery, when in the first day it was almost 20% and in the second day just 6%. Similar with this aspect is the weight variation of the Formicida, when the value was lower in the first day than in the second one.

Table 2. The weight and frequency of the preys.
Tabel 2. Ponderea şi frecvenţa prăzilor.

	WEIGHT				FREQUENCY			
Locality	**St. Ap. Andrei Monastery**			**Cocoş Mon.**	**St. Ap. Andrei Monastery**			**Cocoş Mon.**
Date	**August 8**	**August 9**	**TOTAL**	**August 25**	**August 8**	**August 9**	**TOTAL**	**August 25**
Nematoda	0.13	-	0.05	-	5.26	-	3.23	-
Anelida-Oligocheta	0.38	0.17	0.26	-	5.26	16.67	9.68	-
Crustacea-Izopoda (t)	3.31	0.35	1.55	-	52.63	25.00	41.94	-
Arahnida-Araneida	1.27	0.09	0.57	7.94	31.58	8.33	22.58	28.6
Arahnida-Pseudoscorpionida	-	-	-	0.82	-	-	-	5.35
Arahnida-Opilionidae	-	-	-	0.54	-	-	-	1.78
Myriapoda-Chilopoda	2.04	-	0.83	0.27	42.11	-	25.81	1.78
Myriapoda-Diplopoda	0.13	0.26	0.21	-	5.26	25.00	12.90	-
Collembola	-	-	-	0.27	-	-	-	1.78
Ortoptera	0.38	0.35	0.36	-	15.79	33.33	22.58	-
Dermaptera	1.40	0.17	0.67	-	42.11	16.67	32.26	-
Mantodaea	-	-	-	0.27				1.78
Heteroptera (t)	6.23	2.79	4.19	6.84	78.95	66.67	74.19	28.57
Homoptera-Cicadina	0.76	-	0.31	0.54	10.53	-	6.45	3.57
Lepidoptera (L)	0.13	-	0.05	1.09	5.26	-	3.23	5.35
Lepidoptera	1.02	0.17	0.52	-	31.58	8.33	22.58	-
Trihoptera (L)	0.13	-	0.05	-	5.26	-	3.23	-
Coleoptera-undet.(L) t	0.51	-	0.21	-	15.79	-	9.68	-
Coleoptera-undet	4.45	0.96	2.38	1.64	52.63	5-	51.61	8.92
Coleoptera-Carabidae	17.68	6.10	10.81	12.10	89.47	91.67	90.32	42.90
Coleoptera-Cryzomelidae	-	0.09	0.05	0.27	-	8.33	3.23	1.78
Coleoptera-Coccinelidae	0.64	0.09	0.31	0.82	10.53	8.33	9.68	5.35
Coleoptera-Curculionidae	2.29	1.13	1.60	-	47.37	41.67	45.16	-
Coleoptera-Elateridae	0.64	-	0.26	-	15.79	-	9.68	-
Coleoptera-Stafilinidae	0.13	-	0.05	0.54	5.26	-	3.23	3.57
Coleoptera-Scarabeidae	-	-	-	0.54	-	-	-	3.57
Coleoptera-Lampyridae	0.13	-	0.05	-	5.26	-	3.23	-
Diptera-Nematocera	-	0.09	0.05	6.02	-	8.33	3.23	21.40
Diptera-Brahicera (L)	0.13	0.09	0.10	-	5.26	8.33	6.45	-
Diptera-Brahicera	0.25	0.09	0.16	4.38	5.26	8.33	6.45	23.20
Hymenoptera-undet.	0.13	-	0.05	0.54	5.26	-	3.23	3.57
Hymenoptera-Formicidae	55.73	86.93	74.25	41.60	84.21	10-	90.32	48.20
Hymenoptera-Apidae	-	0.09	0.05	0.27	-	8.33	3.23	1.78
Hymenoptera-Vespidae	-	-	-	0.27	-	-	-	1.78

The frequency of the preys presents lower values than the weight of the same taxa. Regarding St. Ap. Andrei Monastery, after the Formicida appear some taxa with very low weight, such as the Carabida, Isopoda or Curculionida. In comparison with this habitat, the population from Cocoş Monastery presents a much lower frequency of all of the taxa. Moreover, the appearance of the Carabida with a very high frequency is interesting.

Beside the animal prey, we also observed the presence of the vegetal and mineral fragments. A very interesting fact appeared at St. Ap. Andrei Monastery regarding the vegetal remains. Thus, their value was double on the first day than on the second one. Parallel to this situation, the minerals appear with a higher frequency on the second day. In comparison to this habitat, at Cocoş Monastery a much lower value of the two elements was registered.

The food diversity is connected with the feeding intensity. Therefore, St. Ap. Andrei Monastery registers a very high intensity, but the Shannon Wiever index is higher at Cocoş Monastery. Beside this, we can also observe that the food similarity is lower where the diversity is higher.

In order to estimate the differences between the trophic spectrum of the two populations we performed the Mann Whitney test (U test), from which we can state that the differences are valuable between these two habitats ($p<0.05$, $p=0.009$).

DISCUSSIONS

After HUSSEIN & DARWISH (2000) the environmental factors greatly influence the development of the amphibians, factors such as temperature, food density and accessibility. In our case, the very high rate of the feeding activity underlines the presence of very good feeding conditions. However, some *Epidalea viridis* individuals appear at Cocoş Monastery with an empty stomach. This fact can also be connected with the much lower feeding intensity, which suggests that this habitat is less favourable regarding the feeding. The reduced frequency of empty stomachs in the case of the amphibians has been encountered in many situations (COVACIU-MARCOV et al., 2002a, 2003b; CICORT-LUCACIU et al., 2005b). According to some authors, the lack of empty stomachs at a population indicates a positive energy balance (HUEY et al., 2001).

The very high feeding intensity at the habitat from St. Ap. Andrei Monastery is caused by the presence of very small preys, Formicida, which were consumed in high numbers by the frogs. Their amount also influenced the weight value of other preys. Thus, their weight was lower on the first day, when other preys with important value also appeared (Coleoptera, Heteroptera). While the weight of the Formicida was much higher on the second day, this determined the much lower weight of the other preys. The lower amount of the Formicida on the first day can be attributed to the higher humidity of the environment.

The Formicida from Cocoş Monastery appear with a lower weight, in comparison, other typical forest preys are also registered in this habitat (Araneida, Brahicera). This biotope has a stronger connection with the forest, practically these larger preys replacing the Formicida. Due to this cause, the number of consumed preys is also smaller. The spiders and flies, which are larger-sized, have the necessary nutritive value to satisfy the energetic needs of the frogs.

The very high Formicida consumption is characteristic to the *Epidalea viridis* species, because the formic acid is also used to secrete its venom (JONES et al., 1999; BONANSEA & VAIRA, 2007). This fact suggests that the feeding of these frogs is selective, consuming the preys that are more advantageous for their survival.

Together with the smaller-sized preys, there are also larger-sized ones, their weight varying on the habitat. This fact insinuates that the feeding of these frogs is not selectively made regarding on prey-size, but depending on their accessibility, respectively on the advantages that they present.

Some preys appear only in the habitat from St. Ap. Andrei Monastery, for example the Oligochetae, their weight being higher on the first day. This fact can be due exclusively to the higher air humidity after it had rained. Their appearance only in this biotope can also be explained through the presence of several agricultural fields. On the other hand, at Cocoş Monastery, the study was made after more than two weeks after the first study, this being a droughty period, the low environmental humidity not being favourable for the worms.

The weight of a prey does not correspond with its frequency. This fact can firstly be observed at the Curculionida and Carabida. These have a very low weight, while their frequency is very high. This fact suggests that because of the larger size of these Coleopterans, having a higher nutritious value, are consumed in a lower number, but by many frogs. The Coleopterans are also considered very important preys in the case of other amphibian species (COVACIU-MARCOV et al., 2005a; SAS et al., 2005b; HODAR et al., 1990).

Following the Mann Whitney test, we can state that the differences between the two populations are important. Firstly, in the case of the Cocoş Monastery habitat, preys that are characteristic to the forest appear (Araneida, Pseudoscorpionida, Brahicera), because of the strong connections of this habitat with the woods. Regarding the biotope from St. Ap. Andrei Monastery, very different preys appear, being represented by the Coleoptera Carabida, Heteroptera, terrestrial Isopoda, Anelida, therefore preys that are also found outside the abundant vegetation areas. The differences noticed in the weight of the preys can indicate the fact that the feeding methods of the frogs vary depending on the prey density (ÇIÇEK & MERMER, 2006).

The variation of the vegetal frequency is very interesting at St. Ap. Andrei Monastery. This fact is due to the weather conditions, which also influenced the invertebrates' accessibility. On the first day, the frog collecting was made after it had rained. This determined the potential preys to be the ones from the surface of the plants, thus the vegetal fragments being accidentally swallowed together with the pursued prey (STEBBINS & COHEN, 1995). While others consider that the remains help to crush the insects' exoskeleton (EVANS & LAMPO, 1996), or constitute an additional water source (ANDERSON et al., 1999). This fact is in connection with the low frequency of the minerals, which can also be regarded as accidentally swallowed, not having a nutritious content. On the second day, we can already observe the presence of several preys that prefer dryness (Formicida), simultaneously with the decrease of the vegetal frequency and the increase of the mineral frequency. In concordance with this fact, we can explain the appearance of some aquatic preys on the first day.

The diversity is higher in the habitat from Cocoş Monastery, despite the fact that the feeding intensity is lower. Firstly, the very high number of consumed Formicida determines the decrease of this value. On the other hand, this difference can also be explained by the fact that the habitat from Cocoş Monastery, having more abundant vegetation,

also offers potential preys from their surface, but also from areas with poorer vegetation. Even if less taxonomic groups appear in this habitat, they have been consumed on a well-balanced scale, being fewer but ingested by many individuals, which also influences the diversity value. In comparison to another study upon the trophic spectrum of the *Epidalea viridis* species, where H= 2.11 (COVACIU-MARCOV et al., 2005), we can observe that in our case this value is relatively low. In addition, in the case of the mentioned study, we can notice a fluctuation of the diversity, the lowest values being registered in summer. In the case of other amphibian species, the value is higher (SAS et al., 2005c; COGĂLNICEANU et al., 2000b).

The similarity between the individuals is lower when the diversity is higher. This fact is easily understood, because the higher is food diversity in the case of a population, the less are the chances that any two individuals have similar food.

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Ferenți Sára, Cupșa Diana, Dimancea Nicoleta, Filimon Aniela,
University of Oradea
Str. Universităţii, No.1, 410087, Oradea, Romania
E-mail: ferenti_sara@yahoo.com
E-mail: nicoleta_dimancea@yahoo.com
Bogdan Horia Vlad
„Babeş-Bolyai" University,
Faculty of Biology and Geology, Cluj Napoca, Romania

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